WITH WHOM TO NURSE?
CONDITIONS AND CONSEQUENCES OF FEMALE SOCIAL PARTNER CHOICE IN WILD HOUSE MICE

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Andrea Weidt

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Prof. Dr. Marta Manser

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Andrea Weidt

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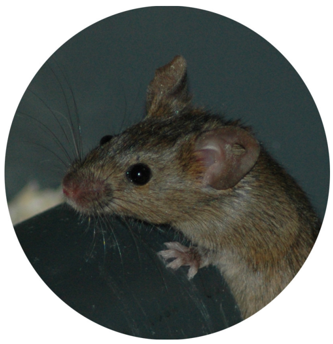

‘THESE CREATURES YOU CALL MICE, YOU SEE, THEY ARE NOT QUITE AS THEY APPEAR. THEY ARE MERELY THE PROTRUSION INTO OUR DIMENSION OF VAST HYPERINTELLIGENT PAN-DIMENSIONAL BEINGS. THE WHOLE BUSINESS WITH THE CHEESE AND THE SQUEAKING IS JUST A FRONT.’

Douglas Adams, 1979; ‘The Hitch Hiker’s Guide to the Galaxy’

CONTENTS

SUMMARY

In numerous species mate choice proved to influence a female's fitness. However, females may also choose partners in other contexts. In group-living animals, females predominantly interact with same-sex conspecifics, and cooperative relationships are frequently established. Females may then be choosy and develop preferences for specific cooperation partners. Yet, female social partner choice has received little attention in the past years, and information on the mechanisms and consequences of these decisions is scarce.

In my thesis, I investigated female social partner choice in communally nursing wild house mice (*Mus domesticus*). Such cooperation requires a relationship where both partners successfully reproduce and wean pups. Therefore, the choice of an appropriate social partner is likely to play a crucial role.

I combined experimental data from indoor enclosures with observations on a population of free-ranging wild house mice in a barn to examine social partner choice decisions. I specifically investigated the occurrence and consequences of social partner choice and the potential for social selection to take place. Additionally, I was interested in the impact of male presence on shaping the social structure among female group members, thus influencing social partner decisions. Furthermore, I investigated possible constraints on female decisions and potential mechanisms involved in the establishment of female cooperative relationships.

Females were choosy when selecting social partners in an enclosure experiment, and such social partner choice entailed fitness benefits. Females kept with a preferred female partner had a significantly higher reproductive success than females kept with a non-preferred partner. Moreover, females were also choosy in a free-ranging population. Even though sharing nesting sites was a prerequisite for cooperation, it could, however, not explain the establishment of individual preferences. This indicates that communal nursing is not a by-product of sociality. Instead, a crucial factor determining communal nursing decisions appeared to be the availability of appropriate social partners. Importantly, the presence of males significantly altered female preferences for social partners. This finding emphasizes the interplay of female-female and female-male interactions, and the necessity to investigate partner choice decisions in the presence of both sexes. Despite reproductive competition, there was no evidence that group-living induces long-lasting stress-responses, even among unfamiliar and unrelated females. Furthermore, females did not appear to maximize benefits and minimize costs of cooperative relationships by coordinating their estrous cycles, and there was no indication that females choose social partners according to MUP (Major Urinary Protein) patterns.

Overall, I could show that in house mice not only mate choice matters. Partner choice also occurs among female group members and may just as well be subject to social selection processes and drive the evolution of female traits.

ZUSAMMENFASSUNG

Etliche Studien haben gezeigt, dass die Wahl eines geeigneten Paarungspartners erhebliche Fitnesskonsequenzen für die wählenden Weibchen haben kann. Weibchen wählen jedoch auch in anderen Zusammenhängen. In Gruppen lebende Weibchen interagieren vorwiegend mit gleichgeschlechtlichen Gruppenmitgliedern, wobei häufig kooperative Beziehungen etabliert werden. Dabei verhalten sich Weibchen durchaus wählerisch und zeigen Präferenzen für spezifische gleichgeschlechtliche Gruppenmitglieder. Allerdings hat die Wahl von Sozialpartnerinnen in den vergangenen Jahren kaum Beachtung gefunden und Information über Mechanismen und Konsequenzen dieser Partnerentscheidungen sind rar.

Diese Arbeit beschäftigt sich mit der Sozialpartnerwahl weiblicher Hausmäuse (*Mus domesticus*), welche ihre Jungtiere gemeinschaftlich säugen. Eine solche Kooperation setzt eine Beziehung voraus, in der beide Partnerinnen erfolgreich Nachkommen aufziehen können. Die Wahl einer geeigneten Sozialpartnerin sollte daher von grosser Bedeutung sein.

Um diese Partnerentscheidungen zu untersuchen, habe ich experimentelle Daten aus Innengehegen mit Beobachtungen an einer Freilandpopulation kombiniert. Zunächst hab ich das Auftreten und die Konsequenzen der Sozialpartnerwahl untersucht. Des weiteren war ich am Einfluss der Männchen auf die Strukturierung der Weibchensozietäten und auf das Wahlverhalten der Weibchen interessiert. Zusätzlich habe ich noch mögliche, das Wahlverhalten einschränkende und beeinflussende Faktoren und Mechanismen betrachtet.

Es zeigte sich, dass Weibchen auch in Bezug auf Sozialpartnerinnen wählerisch waren, und dass eine derartige Wahl Fitnesskonsequenzen hatte. Weibchen, die mit einer präferierten Partnerin gehalten wurden, zeigten im Experiment einen signifikant höheren Fortpflanzungserfolg als Weibchen mit einer nicht-präferierten Partnerin. Die Weibchen waren dabei sowohl unter experimentellen Bedingungen, als auch in der freilebenden Population selektiv. Obwohl die gemeinsame Nutzung von Nistplätzen eine Vorraussetzung für die Kooperation war, konnte dies nicht die Entstehung von Partnerpräferenzen erklären. Dies ist ein eindeutiger Hinweis darauf, dass kooperatives Säugen kein unvermeidbares Nebenprodukt des Gemeinschaftslebens ist. Ein entscheidender Faktor für das Wahlverhalten schien die Verfügbarkeit geeigneter Sozialpartnerinnen zu sein. Interessanterweise modifizierte die Anwesenheit von Männchen die Präferenzen der Weibchen für Sozialpartnerinnen. Diese offensichtliche Wechselwirkung zwischen inner- und zwischengeschlechtlichen Interaktionen verdeutlicht die Notwendigkeit, Wahlentscheidungen in Anwesenheit beider Geschlechter zu untersuchen. Obwohl Weibchen um Paarungsmöglichkeiten konkurrieren, führte das Gruppenleben nicht zu anhaltenden Stressreaktionen, selbst unter nichtbekannten und nichtverwandten Weibchen. Es gab keine Hinweise darauf, dass Weibchen ihre Reproduktionszyklen koordinieren, um die Vorteile der Kooperationsbeziehung zu maximieren und die Kosten zu minimieren. Die Wahl einer Partnerin schien dabei auch nicht von Ähnlichkeiten in Expressionsmustern bestimmter Eiweisse im Urin, so genannter MUPs (Major Urinary Proteins), bestimmt zu sein.

In dieser Arbeit konnte ich am Beispiel der Hausmaus zeigen, dass nicht nur die Wahl eines Paarungspartners von Bedeutung ist. Partnerwahl findet ebenso zwischen Weibchen statt und kann auch dort Selektionsprozessen unterliegen.

INTRODUCTION

INTRODUCTION

'A primary tenet of modern theory on the evolution of social behavior is that an individual's lifetime fitness is affected both by its own actions and by the actions of those with whom it interacts (…). Individuals should (therefore) not only choose what behavior to invoke in a given situation, but also with whom to interact.'

Dugatkin & Sih, 1995

How and why individuals choose partners has been lively discussed in the past decades in the context of female mate choice (Bateson 1983; Andersson 1994). In fact, studies on the mechanisms and consequences of females choosing mating partners are one of the most active areas in the field of behavioural ecology, and are carried out on a variety of taxa, including humans (e.g. Jennions & Petrie 1997; Candolin 2003; Kokko et al. 2003; Geary et al. 2004). Several studies have shown, that mate choice may have tremendous consequences for the females' fitness by either direct benefits, such as access to food, nesting sites, provisioning of paternal care or defence against infanticide, or by genetic benefits such as enhanced immunocompetence of young or 'good genes' passed on to the next generation (e.g. Reynolds & Gross 1990; Barber et al. 2001; Head et al. 2005).

Choosing a mate, however, is not the only context in which females select a partner (Dugatkin & Sih 1995). In group-living animals, each individual female is, besides being in contact with one or multiple males, also surrounded by and in daily interaction with female conspecifics. Such same-sex group members may also serve as potential partners, for example during cooperative activities. In fact, cooperation among female group members is frequent in animal societies and may range from communal foraging or hunting to communal nest defence and cooperative breeding (e.g. Packer & Pusey 1997; Packer et al. 2001; Bosque & Molina 2002; Newton-Fisher 2006). Especially cooperation in the context of reproduction is likely to be accompanied with considerable fitness consequences, and social partner choice is thus expected to be particularly important. Such reproductive cooperation may appear, for example, as joint nesting or the sharing of brood rearing duties (Vehrencamp 1977; Willmer 1985; Bernasconi & Strassmann 1999; Öst et al. 2003). An extreme case of cooperative care among breeding females is communal nursing of young, where female mammals share their milk between own young and offspring produced by another mother (Hayes 2000), a costly activity (e.g. Fuchs 1982; König & Markl 1987; König et al. 1988). Although the frequency of communal nursing is rather low in general (Packer et al. 1992), it occurs in many rodents (reviewed in Hayes 2000), including the study species of this thesis, the house mouse (Sayler & Salmon 1969, 1971; Wilkinson & Baker 1988; König 1994a; Manning et al. 1995; Dobson et al. 2000). It has been suggested that an important factor of female cooperation may be social partner choice (Öst et al. 2005).

Indeed, social relationships vary in quality among female group members. Individual females interact and bond with certain same-sex conspecifics more than with others (e.g. Cords 2002), and such close relationships are frequently linked to cooperative behaviour patterns. Thus, female preferences for social partners in cooperative relationships appear to be non-random. Female partner preferences are frequently reflected by - and thus assessed using - spatial association patterns (e.g. Wilkinson & Baker 1988; Dobson et al. 2000; Stoinski et al. 2003; Smith et al. 2007). In many cases, cooperation partners are closely related individuals (Queller & Strassmann 1998; Griffin & West 2003), but other criteria than kin, such as social rank (Smith et al. 2007), phenotypic cues (e.g. Willmer 1985; Öst et al. 2003), or reciprocal behaviour (Wilkinson 1984) might be as well used in choosing a social partner.

Female group members, however, are not only potential cooperation partners, but also competitors over limited resources. Conflicts are therefore inevitable when females form groups (Sterck et al. 1997), despite any adaptive value of group living (Alexander 1974; Emlen & Oring 1977). Among females rivalry predominantly concerns reproduction, where individuals may not only compete for reproductive resources or the opportunity to reproduce, but also for access to mates (Rosenqvist & Berglund 1992; Jennions & Petrie 1997; Cunningham & Birkhead 1998). Such reproductive competition among female group members often emerges as increased intrasexual aggression (e.g. Rusu & Krackow 2004; Palanza et al. 2005; Bebie & McElligott 2006) and is assumed to play an important role in shaping the social structure and spatial distribution among conspecifics (Slagsvold & Lifjeld 1994; Simeonovska-Nikolova 2003; Rusu & Krackow 2004). By this means, female-female competition can also affect individual preferences for potential cooperation partners.

Most important, however, female social partner choice can be, just as female mate choice, a strong evolutionary force with dramatic consequences (West-Eberhard 1979, 1983). This is the case, as not only the choice of a mating partner (sexual selection), but also the choice of a social partner can be a form of ‘social selection’. Social selection is generally defined as selection resulting from social interactions, such as competition over resources, signalling for mates, providing helping behaviour or, as discussed here, choosing partners (Wolf et al. 1999). The potential for such social selection processes exists whenever individual fitness varies as a result of social interactions (Wolf et al. 1999), a likely scenario in cooperative relationships, especially when they concern reproduction. Social selection differs from ecological selection because an individual’s fitness is not determined only by its own phenotype but also by the phenotype of its social partner. Recent models have demonstrated (Moore et al. 1997; Wolf et al. 1999) that the covariance between the phenotypes of the interacting individuals determines whether and how strongly a particular trait experiences social selection. Factors that can generate such interacting phenotypes are relatedness, inbreeding and kin selection. In addition, assortative (non-random) interactions, behavioural modifications (when an individual alters its behaviour based on that of the social partner) or indirect genetic effects (when genes expressed in an individual have phenotypic effects on the other individual) among unrelated interactants can also allow for the opportunity for social selection (for detailed discussion see Frank 1998; Wolf et al. 1999).

Given the strong potential for the evolution of individual traits, it is, in fact, surprising that the topic of female social partner choice received so little attention in the past years. In this thesis I investigated social partner choice decisions in female house mice, which display reproductive cooperation by communally nursing their young.

Study Species: The House Mouse (*Mus domesticus*)

'*Mice are not quite as they appear, you know...*'

(Douglas Adams, 1979, The Hitch Hikers Guide to the Galaxy)

In fact, this may be true. Even though everyone seems to know these wide-spread, grey, little rodents, leaving their marks in pantries and storage rooms, they exhibit some surprising behaviours.

Originally, house mice inhabited the steppes of Eurasia, from where they spread all over the world following human settlement. They are primarily commensal, meaning that they are typically found in association with human habitation. However, they may become feral and inhabit fields or grasslands, at least temporarily, after the destruction of their man-made habitat (Baker & Petras 1986). House mice are opportunistic colonists and they show an amazing flexibility and adaptability in their feeding behaviour, their reproduction and their social system (Bronson 1984), which most likely contributes to the great success of this rodent species (Mackintosh 1981).

The social organization of the house mouse is influenced by a variety of factors, such as the availability of space or population density, and may differ according to the prevailing conditions (Mackintosh 1981). Typically, house mouse populations are divided into small breeding units, so called demes, which are characterized by one territorial male, few, if any, subordinate males and several breeding and non-breeding females (Anderson & Hill 1965; Reimer & Petras 1967; Selander 1970; Lidicker 1976; Mackintosh 1981). Such demes are generally inbred with limited gene flow among them (Anderson 1970; Berry 1986; Dallas et al. 1995). However, especially under crowded conditions, emigration is common (Anderson 1970). Dispersal is largely undertaken by subordinates that may form their own demes if they reach suitable, unoccupied habitat (Mackintosh 1981). Generally, the sex ratio of those dispersers is skewed to males (Lidicker 1976; Gerlach 1996). However, even though females often remain in their natal territory, they may also emigrate occasionally and successfully integrate into other breeding units, where they encounter unrelated and unfamiliar same-sex conspecifics (Anderson & Hill 1965; Bronson 1979; Baker 1981).

Wild house mice have no distinct mating season, and animals can breed throughout the year provided that nutritious food and ample nesting material is available (Berry 1981). Even low temperatures do not seem to generally affect fertility, as shown by the extraordinary example of mice thriving in meat stores kept at -10°C (Laurie 1946). Reproductive cycles are rather short (average 4-6 days, e.g. Allen 1922; Parkes 1928; Drickamer 1992) and receptivity usually prevails for only one night. Mating often continues over the whole receptive period until males eventually ejaculate at the end of the night when the female's estrous is about to end (Rusu & Krackow 2004). Nevertheless, such male mate guarding does not always seem to be successful, as multiple mating was suggested to occur in at least 20% of all estrous cycles (Dean et al. 2006). Females may move freely across male territories and actively approach and mate with territorial males (Reimer & Petras 1967; Lidicker 1976; Pennycuik et al. 1986).

Under favourable conditions young mice are sexually mature at 6-8 weeks and females can rear a litter of 5-7 young every month (Pelikan 1981; Berry & Jakobson 1971, 1975). Among female group members, which compete over both the opportunity to reproduce and the number of offspring weaned, reproduction is not equally distributed (König 1994a, 1994b). An important aspect of female reproductive competition seems to be access to preferred mating partners (Palanza et al. 1994; Rusu & Krackow 2004). According to Drickamer and colleagues (2000; 2003), females are choosy in selecting mates and the occurrence of such individual preferences has fitness consequences. Females mated to preferred males produce more offspring with an enhanced viability and performance than females mated to non-preferred males (Drickamer et al. 2000). Female-female competition for access to mating partners should be especially prevalent, when the number of preferred males are limited and females are receptive at the same time, thus risking to miss fertilization (Rusu & Krackow 2004).

However, as mentioned before, female conspecifics are also potential cooperation partners in house mice. Females belonging to the same breeding group may cooperate by communally nursing their young. About 50 years ago, Southwick (1955) mentioned for the first time that female house mice pool litters in one communal nest. Females, though, do not only pool their offspring in one communal nest, they also nurse them communally, that is, they distribute milk between own and alien young. Since Southwick's first reference, communal nursing behaviour was mentioned and described in various studies in the laboratory, under semi-natural conditions and in the field (Sayler & Salmon 1969; Baker 1981; Wilkinson & Baker 1988; König 1989; Manning et al. 1992a; 1995; Dobson et al. 2000; Dobson & Baudoin 2002). Even among communally nursing mammals, house mice are special in that they are one of the few species, which do not distinguish between own and alien pups, but nurse them indiscriminately, even under restricted feeding conditions (König 1989). Furthermore, females do not allocate their milk according to own litter size but to the size of the communal nest

(Neuhäusser-Wespy & König, personal communication). Considering that lactation is energetically very costly (König et al. 1988), the question arises why female house mice may spent so much energy on alien young.

This puzzle may be resolved by findings of laboratory studies, which point towards the adaptive value of communal nursing behaviour. For both females involved, communal nursing provides direct fitness benefits due to synergistic effects on pup survival and development (König 1994a, 1994b, 1994c; for a recent review see König 2006). Females that reared litters with a familiar sister weaned significantly more pups over a standardized lifetime than females that were forced to breed solitarily (König 1994b). There is evidence that communally nursing females may avoid peak loads of lactation, which usually occur shortly before weaning when pups are 13-16 days old (König & Markl 1987), given that their litters are several days apart in age (for details see König 2006). Indeed, females seem to benefit from such cooperative reproductive interactions, which presuppose the existence of an egalitarian relationship, where two females successfully reproduce and wean young. To achieve such an egalitarian relationship and thus be able to benefit from cooperation, choosing an appropriate social partner is expected to be crucial.

In fact, already Wilkinson & Baker (1988) suggested in their telemetry-study on a population of wild house mice, that females may actively choose nursing partners and that this choice is based on past spatial association. Several studies in semi-natural enclosures confirmed that cooperative breeding associations are usually preceded by spatial proximity, nest sharing and the development of alliances (Manning et al. 1995; Dobson et al. 2000; Hayes 2000; Rusu et al. 2004; Rusu & Krackow 2004). Such spatial associations are known to be strongly kin oriented (Pennycuik et al. 1986; Wilkinson & Baker 1988; König 1994c; Dobson et al. 2000; Rusu & Krackow 2004), which is suggested to be facilitated by either genetic similarity (Manning et al. 1992b) or familiarity as a proxy for relatedness (Rusu et al. 2004). In addition, preliminary data from a pilot study point towards a potential role of Major Urinary Proteins (MUPs), specifically the sharing of MUP profiles, in displaying social partner preferences (Rusu & Krackow, personal communication). However, the question remains whether free female choice for social partners occurs. Indeed, displaying social partner preferences may be strongly confined as suggested by a study under semi-natural conditions, where communal nursing appeared to be an unavoidable by-product of females sharing same nesting sites (Manning et al. 1995; Hayes 2000).

Overall, there are clear indications that female house mice do not only display partner preferences in the context of mate choice, but also concerning the choice of cooperation partners for communal nursing activities. However, the questions remain whether such social partner preferences indeed result in fitness consequences (and are thus subject to social selection processes), how both competitive and cooperative relationships among female group members impact the social structuring of house mouse groups and therefore shape partner choice decisions, and to which extend females can express social partner choice for communal nursing companions when living in natural house mouse demes.

Thesis Outline: On Fitness Consequences, Competition and Female Decisions

Although social partner choice is likely to be a crucial aspect of reproductive cooperation among female group members, information on this topic is rather scarce. In the following chapters of my thesis, I examine the consequences of social partner choice and the potential for social selection to take place, the impact of male presence on partner choice decisions, and the factors influencing communal nursing decisions in female house mice. I additionally discuss two potential mechanisms involved in partner choice decisions.

In **Chapter 1,** I verify that female house mice were indeed choosy and displayed non-random preferences for specific same-sex group members, and that this social partner choice yielded significant fitness benefits. In an experimental setting, I compared the reproductive behaviour of females kept with either a preferred or a non-preferred social partner over an experimental life-span of half a year. Females in pairs with a preferred partner had a significantly higher probability to give birth and to establish an egalitarian, cooperative relationship, resulting in higher reproductive success than females in non-preferred pairs. Clearly, not only mate choice matters. Also female-female interactions can be subject to social selection processes, driving the evolution of female traits.

The impact of male presence on female social partner choice is demonstrated in **Chapter 2**. Males, however, did not influence female partner decisions by eliciting female mate competition, as expected, but by changing the females' preference. In group living females, competition for mating partners is assumed to play an important role in shaping the spatial distribution and social structure, and thus to influence social partner relationships among female group members. To test the impact of female-female competition on social partner choice, I experimentally compared the frequency of agonistic interactions, the development of social hierarchies, stress hormone production and social partner preferences in groups of unrelated, unfamiliar females in the absence and in the presence of males. There was, however, no indication that male introduction elicited competition among female group members. I found no effect of male presence on female aggression, the development of social hierarchies, stress hormone production and on the females' sociability. Nevertheless, male introduction significantly altered female preferences. This finding also highlights the necessity to consider male presence in studies on female social partner choice in the context of reproductive cooperation.

In **Chapter 3**, I show that females were indeed able to express social partner choice and consequently communal nursing decisions, even under natural conditions, where ecological and social constraints of group life are at work. Preliminary evidence on house mice in semi-natural enclosures suggested that social partner choice is strongly constrained by effects of group living, where communal nursing appeared to be a by-product of sharing nesting sites. I therefore investigated spatial associations and the option of communal nursing in a population of free-ranging wild house mice. Female house mice did not use each option to nurse communally, and communal nest sharing did not inevitably lead to communal nursing activities when nesting partners reproduced at the same time. Interestingly, population density did not influence a females' probability to nurse communally. The decision to cooperate though increased significantly with the number of potential nursing partners, suggesting that the availability of appropriate cooperation partners is crucial and strongly determines communal nursing decisions.

In **Chapter 4**, I question whether potential communal nursing partners minimize competition and maximize benefits by actively coordinating their reproductive cycles. When reproductive cycles of cooperation partners are not fully synchronized, first, mate competition may be reduced, and second, communally nursing females may avoid peak loads during lactation given that litters are several days apart in age. I analyzed vaginal smears and characterized cycle phases of virgin house mouse sisters in different experimental settings in the absence and presence of males. Overall, there was no indication that prospective cooperation partners coordinated their reproductive cycles to minimize competition and maximize energetic benefits. Most probably, the selective pressure on females for actively coordinating their reproduction is very low. Rather than actively coordinating, females may choose appropriate cooperation partners according to their reproductive status. Furthermore, other cues than reproductive synchrony, such as kin-related characteristics, or cues signalling reproductive activity are most likely of higher importance in social partner choice.

Finally, in **Chapter 5**, I investigated the potential of Major Urinary Proteins (MUPs) as a possible cue in female social partner choice. MUPs are a class of highly heterogeneous proteins in the mouse urine, which carry species-, sex-, and individual-specific information. Preliminary data suggest that MUP-profile similarity is associated with female-female preference formation. To test this assumption, I compared dyadic association indices with proportions of MUP-profile sharing (defined by isoelectric focusing) in groups of unfamiliar and unrelated female house mice in semi-natural enclosures. I found no indication that MUP-profile sharing is used as a cue in female social partner choice. However, due to methodological problems these results should be handled with caution.

General Conclusions: Choosy Females & Influential Males

In this thesis I showed on the example of the house mouse that not only mate choice matters. Females also displayed non-random preferences for same-sex social partners, and such female choice entailed fitness benefits. Partner decisions among females are therefore a likely subject to social selection processes, driving the evolution of female traits. Even under more natural conditions, where ecological and social constraints of group life are at work, were female house mice choosy and carried out social partner preferences. I could show that even though sharing nesting sites was a prerequisite for cooperation, it could, however, not explain the establishment of individual preferences. Overall, the availability of appropriate partners seemed to be a crucial factor strongly determining communal nursing decisions. Importantly, female social partner decisions were not independent of intersexual interactions. In fact, male presence influenced the structuring of female societies, surprisingly though not by eliciting competition among female group members, but by altering the females' preferences for social partners. This finding emphasizes the interplay of female-female and female-male interactions and the necessity to investigate partner choice decisions in the presence of both sexes.

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CHAPTER 1

Not only mate choice matters:

Fitness consequences of social partner choice in female house mice

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Not only mate choice matters: Fitness consequences of social partner choice in female house mice

ANDREA WEIDT, SARAH E. HOFMANN, BARBARA KÖNIG

Institute of Zoology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

In addition to sexual selection, selection resulting from social interactions in contexts other than mating can be a potent evolutionary force. Such social selection processes are facilitated whenever individual fitness varies as a result of any form of social interactions. The choice of social partners for communal care of young is such a situation in which interactants potentially experience fitness variance. In this study, we investigated the existence and impact of female social partner choice and the potential for social selection to occur in the cooperatively breeding wild house mouse (*Mus domesticus*). We analysed patterns of individual associations in groups of females, and compared the reproductive behaviour of females grouped with either a preferred or a non-preferred social partner over an experimental life-span of half a year, using spatial association as a measure of preference. We predicted low reproductive competition among preferred social partners and high competition, reflected in lower reproductive success, among non-preferred. Our results showed that female house mice displayed non-random preferences, and that social partner choice yielded significant fitness benefits. Females in pairs with a preferred partner had a significantly higher probability to give birth and to establish an egalitarian, cooperative relationship, resulting in higher reproductive success than females in non-preferred pairs. This suggests that interactions among females are subject to social selection processes, driving the evolution of female traits.

Keywords

Competition, cooperation, female choice, female preference, fitness consequences, house mouse, *Mus domesticus*, reproductive success, social partner choice, social selection

INTRODUCTION

Female mate choice is a well studied topic in evolutionary biology supported by considerable evidence that the choice of a mating partner influences a female's fitness (e.g. Bateson 1983; Andersson 1994; Jennions & Petrie 1997; Drickamer et al. 2000, 2003; Kokko et al. 2003). In contrast, female social partner choice and its impact on fitness have not received much attention. This is surprising, as both sexual selection and social partner choice are forms of 'social selection' (that is selection resulting in general from social interactions; West-Eberhard 1979, 1983) that can have profound effects on fitness. Social selection is a specific case of natural selection in that an individual's fitness is not only determined by its own phenotype but also by the phenotype of its social partner. The potential for social selection therefore exists whenever individual fitness varies as a result of social interactions (Wolf et al. 1999). Recent models have demonstrated (Moore et al. 1997; Wolf et al. 1999) that the covariance between the phenotypes of the interacting individuals determines whether and how strongly a particular trait experiences social selection. Factors that can generate such interacting phenotypes are relatedness, inbreeding and kin selection. In addition, assortative (non-random) interactions, behavioural modifications (when an individual alters its behaviour based on that of the social partner) or indirect genetic effects (when genes expressed in an individual have phenotypic effects on the other individual) among unrelated interactants can also allow for the opportunity for social selection (for detailed discussion see Frank 1998; Wolf et al. 1999).

Female social partner choice, for example during reproductive cooperation, may result in interactants experiencing fitness variance. Reproductive cooperation appears as joint nesting and sharing of brood rearing duties (Vehrencamp 1977; Willmer 1985; Bernasconi & Strassmann 1999; Öst et al. 2003), and may even extend to lactation of non-offspring (young produced by another mother), a costly activity (e.g. Fuchs 1982; König & Markl 1987; König et al. 1988). Such communal nursing behaviour is common in many rodent species (reviewed in Packer et al. 1992; Hayes 2000) including the house mouse (Sayler & Salmon 1969, 1971; Wilkinson & Baker 1988; König 1994a; Manning et al. 1995; Dobson et al. 2000), and offers an ideal setting to study the existence and impact of female social partner choice on fitness and the potential for social selection to occur.

Wild house mice, *Mus domesticus*, live in groups that are typically characterized by one territorial male, few, if any, subordinate males and several breeding and non-breeding females (Anderson & Hill 1965; Reimer & Petras 1967; Selander 1970; Lidicker 1976; Mackintosh 1981). Females often remain in their natal territory, but occasionally disperse and successfully immigrate into other breeding units, where they encounter unrelated and unfamiliar same-sex conspecifics (Anderson & Hill 1965; Bronson 1979; Baker 1981). In house mouse societies, reproduction is unequally distributed among females, which compete over both the opportunity to reproduce and the number of offspring weaned (König 1994a, b). Communal nursing, characterized by an egalitarian relationship of two reproducing females, is suggested to provide direct benefits for both partners (for a recent review see König 2006). However, to achieve such a mutualistic relationship and thus to benefit from cooperation, choosing the right social partner may be crucial. In the context of mate choice, the occurrence of individual preferences and their impact on fitness, offspring viability and progeny performance has already been intensely studied in house mice (Drickamer et al. 2000, 2003; Gowaty et al. 2003).

We predicted that females also establish non-random associations when given the choice between several female partners, and that choice has fitness consequences. To test this prediction, we analysed the patterns of individual associations in groups of females, and compared the reproductive success of females experimentally grouped with either a preferred or a non-preferred social partner, assuming that females should prefer conspecifics with whom they have low competition for reproduction. We thus hypothesized that individual females in pairs of preferred partners have a higher probability to reproduce and successfully wean pups and show an earlier onset of reproduction, resulting in an overall higher reproductive success compared to females in pairs of non-preferred partners.

We focused on previously unfamiliar and genetically unrelated females, because familiar sisters almost always share the same nest and establish egalitarian reproductive relationships (König 1994c, 2006). Unfamiliar non-sisters, nevertheless, represent a social category a maturing female may encounter in natural house mouse demes, especially following migration. Social partner choice, if advantageous, should be most prominent under such environmental conditions.

METHODS

Animals and Husbandry

Animals were direct descendants of wild-caught and randomly bred house mice, *Mus domesticus*, originating from three wild populations in the vicinity of Zurich, Switzerland (all populations shared the same karyotype, 2n = 24). Mice were housed in Macrolon-III-cages (23.5 x 39 x 15 cm) on standard animal bedding, with food (laboratory animal diet for mice and rats, no. 3804 & 3336, Provimi Kliba SA, Kaiseraugst, Switzerland), water and nest building material ad libitum. Pups were separated from their parents at the age of 23 days and housed with same-sex litter mates.

Throughout the experiment animals were kept under standard laboratory conditions (14:10h light:dark cycle, one hour twilight phase at beginning and end of the light phase; 22 ± 1°C, 50-60% relative humidity).

Enclosures and Cage Systems

Indoor enclosures used in the first phase of the experiment were 7 m^2 in size and surrounded by 80 cm high aluminium walls. Each enclosure was filled with bedding, equipped with six nest-boxes (15 cm diameter, 15 cm height), several PVC barriers for structuring, hay and paper towels as nest building material and three feeding and drinking sites.

Cage systems used in the second phase of the experiment consisted of three Macrolon-II cages (18 x 24 x 14 cm) connected by perspex plastic tubes. Animal bedding, nesting material, food and water were provided in each system.

Experimental Trials

Between November 2004 and September 2006 we conducted 20 trials with six and two trials with five adult virgin females (2-3 months of age), all with two adult, sexually inexperienced males (2-7 months old). The two trials with only five females resulted from the removal of one female prior to the start of the experiment (due to wounds inflicted by conspecifics). In each trial, the females were unfamiliar and genetically unrelated to each other. The males were always unrelated and unfamiliar to the females of the trial. Within a trial, females did not differ more than one month in age and 2 g in weight when they were released into the enclosures. All females were equipped with trovan® transponders (ID 100, TROVAN electronic identification systems) for individual identification. The rice grain shaped transponders with RFID tags encapsulated in bio-compatible glass had a length of 11.5 mm, a diameter of 2.1 mm and weighted 0.1 g, which was at most 0.6% of the animal's body weight. All transponders, which were presterilized and provided in sterile needles, were injected subcutaneously in the neck region, parallel to the backbone, by a licensed person. Animals were not anaesthetized during this rapid procedure and resumed normal behaviour immediately. There was no evidence that transponders migrated around the body.

Eighteen days after releasing females into the enclosures, we placed the two males in separate cages in the middle of each enclosure. The bedding of males was mixed once per week and the positions of the two cages were interchanged several times. The introduction of caged males was intended to signal mating opportunities to the females without enabling them to mate.

At the end of the experiment, all animals were euthanized in their home cages by CO_2 inhalation, as recommended for mice in the guidelines of the Swiss Veterinary Service (http://www.bvet.admin.ch/tierschutz/00919/00940/). CO_2 was continuously and silently induced into the animals' covered-up home cages, which were placed in a separate room, until well after the animals stopped breathing.

Spatial Associations and Grouping of Females: Phase One

During phase one of the experiment, a period of 15 days starting with the introduction of caged males, we collected data on the females' nest-box use. Daily at midday, we registered the females' locations in the nest-boxes with a portable transponder reader (LID 500 Hand-Held Reader, TROVAN electronic identification systems). In addition, we observed all animal groups for the occurrence of aggression each evening. Each animal was weighed and carefully checked for scars or wounds once a week. We used spatial associations prior to reproduction, specifically the sharing of nest-boxes, as a measure of social partner preferences, a relation suggested in several previous studies on house mice (Wilkinson & Baker 1988; Manning et al. 1995; Dobson et al. 2000; Hayes 2000; Rusu et al. 2004). Dyadic associations between females of the same trial were determined over the period of 15 days according to the symmetrical index of Fager (I_{ij}-index, modified by Kerth and König (1999)). We calculated the expected probability that two females of a dyad meet in any of the nest-boxes by chance, and compared this expected value with the observed data using a binomial test. Two females were counted as highly associated and regarded as preferred partners when they shared nest-boxes significantly more often than expected by chance.

Based on the dyadic associations in the presence of caged males, we defined two different treatments: for 'preferred partners', we chose the two females with the highest (significant) association and for 'non-preferred' the two females with the lowest association among the females within each trial. If more than one dyad had the same highest or lowest association values, we selected among them randomly. Preferred and non-preferred dyads were then randomly assigned to one of the trial's two males, and introduced into a cage system. In two trials, all female dyads showed higher than random association values and we did not carry out the non-preferred partner treatment.

Monitoring Reproduction: Phase Two

On the first day in the cage system, the male was separated by a wire mesh in one of the three cages. Thereafter, the two females and the male cohabited for a period of 120 days (phase two of the experiment), reflecting a common experimental lifespan (König 1994a, b, c, 2006).

Newborn pups were registered at daily inspections of the cages. Dead pups were counted and removed. Juveniles were removed from the parental group when 28 days old. Once a week (starting on day 14 of phase two) we measured body weight, carefully checked animals for scars or wounds and monitored female reproduction. Furthermore, groups were daily monitored for intra-group aggression. Whenever aggression resulted in one of the females being wounded, we removed this female from the cage system. Wounded females were not expected to reproduce anymore within the group and to possibly die if not separated (see König 1994b). The other female of the dyad was left with the male for the remainder of the experiment and the dyad entered statistical analysis. Such dyads where separation events have taken place were indicated as pairs with ‘elevated aggression’. The injured female was considered as ‘dead’ and entered data sheets with values of reproductive parameters obtained until removal. Wounds leading to separation events included visible bites on the back, the legs or the tail. All separated animals, which were kept for at least four weeks to ensure that they were not pregnant, recovered and wounds healed within a few days without additional treatment. In one trial, one female in a non-preferred association proved to have crippled genitalia and inner sexual organs, and the pair was therefore excluded from analyses.

We determined maternities of a newborn litter by considering changes in individual females’ body weight and by investigating the females’ teats for signs of lactation. In cases of maternity uncertainties, when weekly body weight differences of a female were marginal or when both females of a dyad gave birth on the same day, we determined unknown maternities by genetic analyses from tissue samples taken post mortem.

We extracted DNA from ear lobe cuts using a salt chloroform method (Müllenbach et al. 1989). We amplified alleles at nine microsatellite loci (D1Mit108, D6Mit390, D7Mit266, D11Mit90, D11Mit150, D13Mit1, D17Mit21, D17Mit87, D19Mit25), characterized in the Mouse Genome Informatics database (Eppig et al. 2005) at www.informatics.jax.org., using 3 multiplex PCRs with 3 loci each. PCR amplifications were carried out with the following cycling protocol: 7 min at 94℃, 30 cycles (31 for one multiplex) of 30 sec at 94℃, 45 sec at 58℃, 1 min at 72℃ and 20 min at 72℃. Fragment analyses were performed using an ABI Genescan system.

We carried out maternity assignments manually by exclusion, by comparing the alleles of the two putative mothers and the known father with the alleles of the respective pups at all loci, assuming Mendelian segregation. In five cases, where pups of a communal litter could not unambiguously be assigned to one of the females, numbers were distributed evenly between the two females of the pair for final analyses.

Partitioning of reproduction

We compared the frequencies that both females of a dyad gave birth to a litter between treatments (preferred versus non-preferred female dyads), using a Fisher’s Exact Test. Furthermore we divided all female dyads into the two categories ‘egalitarian’ (both females weaned at least one pup) and ‘non-egalitarian’ (one or none of the females successfully weaned pups, see König 1994a, c, 2006) and used a Chi-square Test to compare the frequency distributions of egalitarian reproduction and of elevated aggression between treatments.

In egalitarian dyads, we used a Mann-Whitney U Test to compare the dyads' reproductive skew between treatments. Due to our experimental setup, we used the S-index according to Keller & Vargo (1993) and Reeve & Ratnieks (1993) to calculate reproductive skew.

Onset of reproduction

Onset of reproduction was defined as the number of days between access of the male to the females in the cage system, and the birth of the first litter, irrespective of the litter's survival. To analyse whether onset of reproduction differed between treatments, we carried out a linear mixed-effects model (Venables & Ripley 2002) fitted by residual maximum likelihood, using treatment, elevated aggression and their interaction as fixed factors. Trial identity was incorporated as a random term to account for potential similarities of females originating from the same enclosure. We log-transformed the data to meet the assumptions of normality and homogeneity of variances.

In each dyad, the female with most pups weaned during the experimental lifespan was ranked as superior, the partner as inferior. To investigate the relation between the order in which the two females of a dyad began reproducing and the females' final reproductive rank within the dyad, we carried out a generalized linear mixed-effects model using a binomial error structure and the logit link function. The model was fitted using penalized quasi likelihood estimation (for details see Venables & Ripley 2002). We used reproductive rank as the binary response variable, and order of reproductive onset, treatment, and their interaction as fixed factors. We nested treatment within the trial identity as a random term to investigate the relation for each dyad. Dyads in which females started reproducing on the same day (3 cases) or in which both females weaned the same number of pups (1 case) or no pups (9 cases) during the experiment were excluded. This analysis was thus carried out with 16 preferred and 12 non-preferred pairs.

Reproductive success

Reproductive success was calculated by counting the number of a female's pups that reached an age of 17 days, that is when pups survive on their own under laboratory conditions (König & Markl 1987). We used a Mann-Whitney U Test to compare the mean reproductive success of females in a dyad between treatments and the individual reproductive success between superior females, as well as inferior females in both treatments.

Statistical Analyses

Statistical models were carried out using R for Windows, Version 2.2.0 (R Development Core Team 2005) and the packages 'nlme' (Pinheiro et al. 2004) and 'MASS' (Venables & Ripley 2002). Nonparametric statistics were conducted using SPSS 13.0 (SPSS Inc. Chicago, IL, USA). All tests were two-tailed and effects were regarded as significant at $P \leq 0.05$.

RESULTS

Spatial Structuring and Associations

In the 22 trials, 42% of all dyadic associations were significant, meaning that two individual females shared nest-boxes significantly more often than expected by chance, whereas all other dyads (58%) were in the range of random associations. Overall, 72% of the females exhibited significant positive associations to at least one other female during phase one of the experiment. Association indices ranged from zero to one, where an association index of zero indicates that two individuals were never found in the same nest-box, an index of one that two females always shared the same nest-box. Average association indices per trial ranged from 0.1 to 0.49 (*mean* ± SE = 0.28 ± 0.03, $N = 22$).

Female Reproduction

Partitioning of reproduction

Females in preferred partner dyads had a significantly higher probability to reproduce (Fisher's Exact Test: $P = 0.026$) and successfully wean pups (Chi-square Test: $\chi^2_1 = 4.45$, $P = 0.035$) than females in non-preferred ones (Table 1). Communal nursing of pups occurred in all dyads in which both females reared litters that did not differ by more than 17 days in age.

Table 1 Behavioural and reproductive parameters of experimental female dyads[1]

	Preferred pairs ($N = 22$)	**Non-preferred pairs ($N = 19$)**
Number of dyads in which:		
Elevated aggression occurred	7	6
None of the females gave birth to a litter	0	0
One female gave birth to a litter	2	8
Both females gave birth to a litter	20	11
None of the females weaned pups	3	6
One female weaned pups	6	8
Both females weaned pups	13	5
Reproductive skew of egalitarian dyads	0.08 (0.00 / 0.52)	0.18 (0.00 / 0.44)

[1]Behavioural and reproductive parameters are compared between preferred and non-preferred pairs in phase two of the experiment. Reproductive skew of egalitarian dyads (both females weaned pups) is given as median, minimum and maximum.

The frequency of elevated aggression leading to separation events did not differ significantly between treatments (Chi-square Test: $\chi^2_1 = 0$, $P = 0.99$; Table 1). As none of the separated females weaned pups before we removed them from the cage system (between 10 days and four months after the start of phase two) all groups with elevated aggression resulted in non-egalitarian dyads.

In egalitarian dyads, where both females weaned pups, reproductive skew did not differ significantly between preferred and non-preferred partners (Mann-Whitney U Test: $U = 32.0$, $P = 0.96$; Table 1).

Onset of reproduction

Concerning the onset of reproduction, elevated aggression leading to separation events had a significantly different effect on preferred and non-preferred female partners (treatment x elevated aggression: $F_{1,15} = 26.44$, $P < 0.001$; treatment: $F_{1,15} = 7.83$, $P = 0.014$; elevated aggression: $F_{1,15} = 21.24$, $P < 0.001$). Preferred and non-preferred pairs without elevated aggression hardly differed in the time until birth of the first litter (preferred pairs: *median* / IQR = 25 / 5.5 days, $N = 15$; non-preferred pairs: *median* / IQR = 25 / 6 days, $N = 13$; Fig. 1). However, in dyads with elevated aggression where one female had to be removed, the onset of reproduction was delayed in non-preferred pairs (*median* / IQR = 54 / 29.8 days, $N = 6$), but not in preferred pairs (*median* / IQR = 25 / 1 day(s), $N = 7$; Fig. 1).

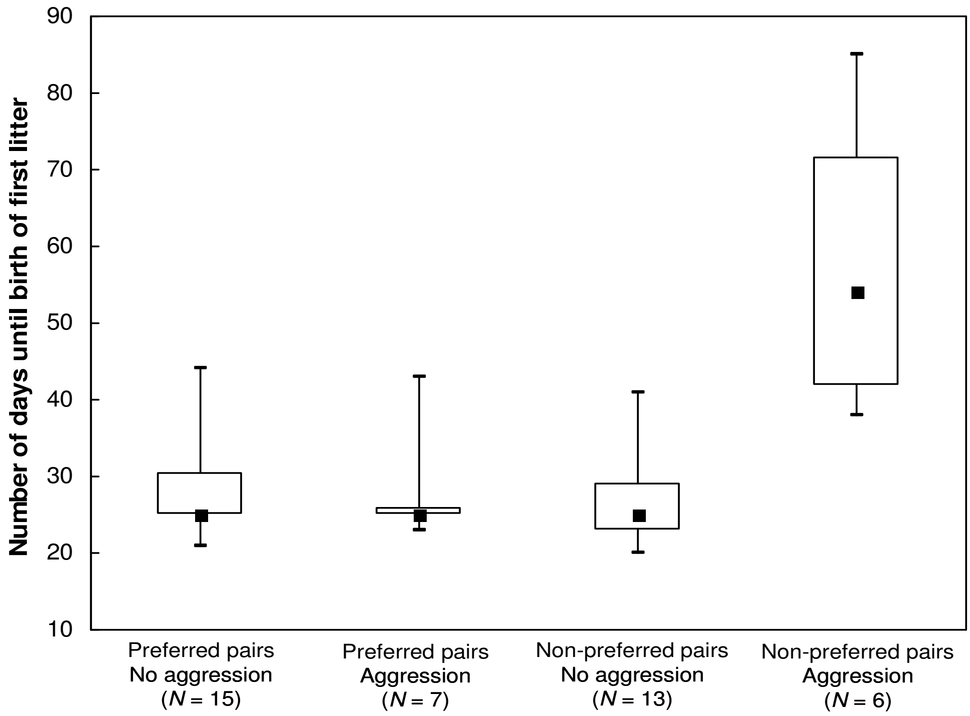

Figure 1 Onset of reproduction in ‘preferred pairs’ and ‘non-preferred pairs’ where no elevated aggression occurred (indicated as ‘no aggression’) and where elevated aggression leading to separation took place (indicated as ‘aggression’). Filled square: median, box: interquartile range 25%-75%, whiskers: Min.-Max..

The order in which females started reproducing and the females' final reproductive rank differed significantly between treatments (first reproducing x treatment: $F_{1,26}$ = 6.77, P = 0.015). The first female that gave birth to a litter had a high probability to be reproductively superior in non-preferred dyads (nine out of 12 females). In pairs with preferred partners, in contrast, no such correlation occurred (six out of 16 females which gave birth first were finally reproductively superior).

Reproductive success

Associations prior to reproduction affected the females' mean reproductive success. Females in preferred pairs had a significantly higher mean reproductive success than females in non-preferred pairs (preferred pairs: *median* / IQR = 9.3 / 4 pups, N = 22; non-preferred pairs: *median* / IQR = 3.5 / 8.8 pups, N = 22; Mann-Whitney U Test: U = 133.5, P = 0.047; Fig. 2).

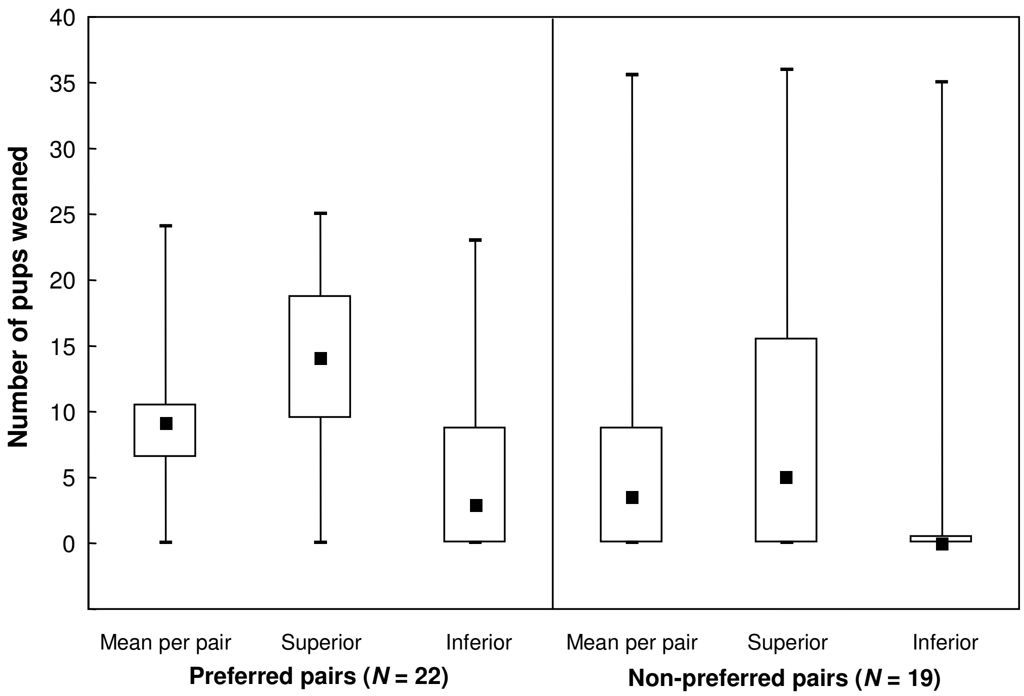

Figure 2 Reproductive success in preferred and non-preferred pairs. The boxes show the mean reproductive success of individual females per pair and the individual reproductive success of the superior females and of the inferior females for both treatments.

For superior females, treatment had no significant effect on the number of offspring weaned during the experimental lifespan (preferred pairs: *median* / IQR = 14 / 9.3 pups, N = 22; non-preferred pairs: *median* / IQR = 5 / 15.5 pups, N = 22; Mann-Whitney U Test: U = 148.5, P = 0.11; Fig. 2). Inferior females in preferred pairs, however, had a significantly higher individual reproductive success than inferior females in non-preferred pairs (preferred pairs: *median* / IQR = 3 / 8.8 pups, N = 22; non-preferred pairs: *median* / IQR = 0 / 0.5 pups, N = 22; Mann-Whitney U Test: U = 136.5, P = 0.037; Fig. 2).

DISCUSSION

Individual females in pairs of preferred partners proved to have a significantly higher mean reproductive success than females in non-preferred pairs, indicating that social partner choice yields fitness benefits in female house mice.

Choosy Females

When kept in all female groups, female house mice were indeed choosy, which is in line with Dugatkin's and Sih's (1995) statement that females do not only display mate preferences, but also preferences for social partners in other contexts than mating. Nearly three quarters of all females exhibited significant associations to at least one other female of the trial. The fact that association indices varied greatly and that less than half of all associations were significant, further supports the conclusion that females discriminated between social partners and displayed individual preferences. Such non-random preferences for social partners are also known from various other species, for example in primates (Cords 2002), bats (Wilkinson 1985) or birds (Öst et al. 2003), and often occur in the context of cooperation.

Female house mice did not only display preferences, but assortative interactions further proved to result in fitness consequences, as already demonstrated in the context of mate choice in house mice (Drickamer et al. 2000, 2003; Gowaty et al. 2003).

Reproductive Competition

Females that were kept with previously preferred social partners had a higher probability to reproduce and to wean pups compared to females living with previously non-preferred partners. This indicates that females generally associated with social partners with whom they had a lower potential for reproductive competition, even though conflicts were not totally absent as apparent in the occasional occurrence of elevated aggression.

With a preferred social partner females had a higher probability to establish egalitarian relationships. Both females could end up as reproductively superior after completion of the experiment, irrespective of the order of first reproduction. In non-preferred dyads however, the first female to reproduce was finally superior in 75% of the cases, whereas the second female did not reproduce at all in over half of those cases, suggesting competition over the order of reproduction. In non-preferred pairs with separation events due to elevated aggression, superior females produced the first litter later than both females in preferred pairs. Additionally, inferior females in such dyads did not produce any litters at all. Competition therefore seems to be mediated by aggression among the females. Even though the frequency of elevated aggression did not differ quantitatively between preferred and non-preferred female pairs, the quality of aggression did, suggesting a different kind of relationship between preferred and non-preferred females. Only inferior females in non-preferred pairs, which had a significantly lower individual reproductive success than inferior females in preferred pairs, suffered fitness costs.

Elevated Aggression

High aggression among females was absent during the 15 days in the enclosures. None of the 130 females had to be removed due to wounds inflicted by conspecifics. After introduction to the cage systems, however, we had to separate 32% of both preferred and non-preferred pairs. Here, space was more confined and females might not have been able to avoid each other, possibly resulting in more frequent aggressive interactions.

Nevertheless, confinement does not explain elevated aggression among preferred females. Interestingly, such elevated aggression occurred with similar frequencies in preferred and in non-preferred pairs. One possible explanation is that high spatial associations may not always reflect social partner choice. This can occur when several females prefer the same partner, resulting in all of them having the same pair wise associations. If then by chance competing females were chosen for phase two of the experiment, erroneous assignment could occur. However, in six out of seven cases where elevated aggression occurred in preferred pairs, the chosen pair unambiguously had the highest pair wise association. Therefore, the probability for false assignment is very low. Another possible explanation is that females alter their partner preferences during different reproductive phases, as shown for example in women (Puts 2006) and toads (Lea et al. 2000). Severe aggression leading to separation did not occur immediately but between 20 days and four months after introducing preferred female partners into the cage systems, suggesting that females were cycling or early pregnant when elevated aggression occurred. This indicates that preferences for social partners might in fact be altered or fine-tuned with changes in the reproductive state.

Cooperative Relationships

Reproductive skew in egalitarian pairs (pairs in which both females weaned at least one pup) did not differ significantly between treatments. This indicates that once females established an egalitarian relationship, the quality of such associations did not differ between preferred and non-preferred partners. The crucial difference between the two treatments refers to the probability to reproduce and successfully wean pups.

Juvenile familiarity improves a female's probability to establish cooperative reproductive relationships with a same sex partner (König 1994c; Rusu et al. 2004). Among familiar females aggression does not occur to such an extent that they have to be separated (König 1994a, c). Here we show that similar cooperative relationships can also be established among unrelated females lacking juvenile familiarity, especially among preferred partners. Preferred females competed less over reproduction and had a higher probability to establish cooperative relationships than non-preferred ones. Despite the fact that elevated aggression occasionally occurred among preferred pairs, our findings demonstrate that juvenile familiarity is no necessity for communal nursing. This allows females to establish successful cooperative relationships with formerly unfamiliar and unrelated partners after emigration from the natal group.

Individual Preferences and Social Selection

Non-random interactions between group-living female house mice resulted in fitness variances. This offers the potential for social selection and for evolution of social partner preferences. If a female house mouse with a specific trait seeks out a social partner based on its phenotype, the result will be covariance between the interactants' phenotypes (see introduction).

Our study did not intend to investigate the mechanisms of, or cues used for individual preferences. Still, such traits may be degree of relatedness or physical or physiological cues that refer to a female's ability to raise pups, as for example body condition or hormone levels. Indeed, females may associate with relatives, as for example in several cooperatively breeding vertebrates (Griffin & West 2003) or in social insects (Queller & Strassmann 1998), but also according to other criteria such as body condition as in female eider ducks (Öst et al. 2003, 2005), body size as in *Cerceris* wasps (Willmer 1985) or previous experience as in the common vampire bat (Wilkinson 1984, 1985). In house mice, MHC-characteristics, specifically MHC-dissimilarity, are important for mate choice (Drickamer et al. 2000; Penn 2002; Gowaty et al. 2003). MHC-characteristics, in this case MHC-similarity (which can be mediated by phenotype matching), may also play a role in choosing social partners. This would be in line with the finding, that females choose partners with low reproductive competition, as expected for individuals that share alleles by common descent. Partner preferences thus might also be self-referential, as has been suggested for mate choice in house mice (Drickamer et al. 2000, 2003; Gowaty et al. 2003). Future studies have to analyse whether female house mice use MHC-characteristics or other physiological cues resulting in assortative traits of social partners.

Clearly not only mate choice matters. Our results showed that females also display non-random preferences for social partners, and that this choice entails fitness benefits. Interactions among females thus may be subject to social selection processes, driving the evolution of female traits. However, the mechanisms of female relationships are rather rarely the focus of scientific interest. We therefore encourage further studies on female-female interactions and their impact on selection.

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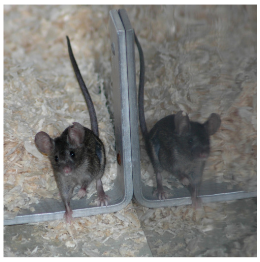
CHAPTER 2



Males make females change their mind!
Impact of male presence on female-female competition and social partner choice in wild house mice (*Mus domesticus*)

submitted

Males make females change their mind! Impact of male presence on female-female competition and social partner choice in wild house mice (*Mus domesticus*)

ANDREA WEIDT[1], SARAH E. HOFMANN[1], LORENZ GYGAX[1,2], CHADI TOUMA[3], RUPERT PALME[4] & BARBARA KÖNIG[1]

[1]Institute of Zoology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
[2]Agroscope Reckenholz-Taenikon Research Station ART, Taenikon, 8356 Ettenhausen, Switzerland
[3]Department of Behavioral Neuroendocrinology, Max Planck Institute of Psychiatry, Kraepelinstrasse 2-10, 80804 Munich, Germany
[4]Institute of Biochemistry, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria

In group living animals, competition for mating partners is assumed to play an important role in shaping the spatial distribution and social structure among female group members. In this study, we investigated the impact of male presence on the development of female-female competition, the structuring of groups and female social partner choice in wild house mice. We predicted that, by eliciting competition, males shape the social structure among female group members and thus affect female preferences for potential cooperation partners. To test this prediction, we compared the frequency of agonistic interactions, social hierarchies, stress hormone production and social partner preferences in groups of unrelated, unfamiliar females in the absence and presence of males. There was no indication that the introduction of males elicited competition among female group members. We found no effect of male presence on female aggression, hierarchies, stress hormone production and on the females' sociability. However, male introduction significantly altered female preferences. Our results therefore indicate that male presence indeed influences the structuring of female societies, however not by eliciting competition among females, but rather by changing female preferences for social partners. Clearly, males make females change their mind, which highlights the necessity to consider male presence in studies on female social partner choice.

Keywords

Competition, female-female relationships, glucocorticoids, house mice, mate competition, *Mus domesticus*, partner preferences, social hierarchies, social partner choice, social structure, stress

INTRODUCTION

Conspecifics are a major environmental factor, in particular for group living animals. From a female's point of view, males may serve as potential mating partners and same-sex conspecifics as social and potential cooperation partners. On the other hand conspecifics are also competitors for limited resources when living in close proximity (Wilson 1975; West-Eberhard 1979). Conflicts are therefore inevitable when females form groups (Sterck et al. 1997), despite any adaptive value of group living (Alexander 1974; Emlen & Oring 1977). Among females, rivalry predominantly concerns reproduction, where individuals may not only compete for reproductive resources or the opportunity to reproduce, but also for access to mates (Rosenqvist & Berglund 1992; Jennions & Petrie 1997; Cunningham & Birkhead 1998).

Female mate competition often emerges as increased intrasexual aggression (e.g. Rusu & Krackow 2004; Palanza et al. 2005; Bebie & McElligott 2006) and is assumed to play an important role in shaping social structure and spatial distribution among conspecifics (Slagsvold & Lifjeld 1994; Simeonovska-Nikolova 2003; Rusu & Krackow 2004). The social structure, particularly spatial associations among certain female group members, is in turn linked to cooperative relationships, as for example shown in primates (Cords 2002), bats (Wilkinson 1985), rodents (Rusu et al. 2004; Weidt et al. in press), birds (Öst et al. 2003) or fish (Croft et al. 2006). Thus, by affecting social structure, female-female competition may influence individual preferences for potential cooperation partners.

Wild house mice, *Mus domesticus*, live in groups that are typically characterised by one territorial male, few, if any, subordinate males and several breeding and non-breeding females (Anderson & Hill 1965; Reimer & Petras 1967; Selander 1970; Lidicker 1976; Mackintosh 1981). Females often remain in their natal territory, but occasionally disperse and successfully immigrate into another breeding unit, where they encounter unrelated and unfamiliar same-sex conspecifics (Anderson & Hill 1965; Bronson 1979; Baker 1981). Female house mice belonging to the same breeding group may cooperate by communally nursing their young (e.g. Sayler & Salmon 1969; Baker 1981; Wilkinson & Baker 1988; König 1989). Thereby, females display non-random preferences for cooperation partners, which yields significant fitness benefits (Weidt et al. in press). At the same time, however, group living females may compete over access to males (Palanza et al. 1994; Rusu & Krackow 2004). To understand the role of female intrasexual competition for the establishment of social associations, we experimentally investigated the impact of male presence on the development of female-female competition, the structuring of female groups, and female social partner choice in communally nursing wild house mice.

We expected that male presence affects social partner preferences of females. Due to the importance of genetic benefits of mate choice (Penn & Potts 1999) males should elicit competition and thus shape female social structure. To test this prediction, we compared behavioural parameters, stress hormone production and social partner preferences in groups of unrelated, unfamiliar females in the absence and presence of males. We specifically hypothesized that male introduction into all-female groups 1) leads to an increase of agonistic interactions between female group members, 2) establishes a dominance hierarchy among females, 3) increases female stress hormone production, and 4) decreases the females' sociability, i.e. reducing the number of association partners.

We focused on previously unfamiliar and genetically unrelated females in this study as they appear to compete most severely for reproduction (Rusu & Krackow 2004; Palanza et al. 2005). Under natural conditions, unfamiliar non-sisters represent a social category, a maturing female may encounter when emigrating from its natal territory. Such females may either enter another group or form a new one with previously unfamiliar und unrelated females (e.g. Baker 1981; Gerlach 1990, 1996).

METHODS

Animal Husbandry & Enclosures

Animals were direct descendants of wild-caught and randomly bred house mice, *Mus domesticus*, originating from three wild populations in the vicinity of Zurich, Switzerland (all populations shared the same karyotype, 2n = 24). Mice were housed in Macrolon-III-cages (23.5 x 39 x 15 cm) on standard animal bedding, with food (laboratory animal diet for mice and rats, no. 3804 & 3336, Provimi Kliba SA, Kaiseraugst, Switzerland), water and nest building material *ad libitum*. Pups were separated from their parents at the age of 23 days and housed with same-sex litter mates.

The experiment was carried out in indoor enclosures, which were 7 m^2 in size and surrounded by 80 cm high aluminium walls. Each enclosure was filled with standard animal bedding, equipped with six nest-boxes (15 cm diameter, 15 cm height), several PVC barriers for structuring, hay and paper towels as nest building material and three feeding and drinking sites. Experimental animals were kept under standard laboratory conditions (14:10 hours light:dark cycle, one hour twilight phase at beginning and end of the light phase, light phase started at 7:30 h.; 22±1°C, 50-60% relative humidity). At the end of this experiment, animals participated in a follow-up study (see Weidt et al. in press).

Experimental Procedure

Between November 2004 and May 2006 we investigated 22 replicate groups, each with six adult virgin females (2-3 months of age) and two adult, sexually inexperienced males (2-7 months old). In each group, females were unfamiliar and genetically unrelated to each other. The males were unrelated and unfamiliar to the females of the replicate. Within a replicate, females did not differ more than one month in age and no more than 2 g in weight at the start of the experiment. All females were equipped with subcutaneously injected transponders (ID 100, TROVAN electronic identification systems) and obtained fur cuts and ear punches for visual individual identification during behavioural observations. Animals were not anaesthetized during these rapid procedures and resumed normal behaviour immediately.

Females of one replicate were simultaneously introduced into the enclosure. During the first 18 days of the experiment the animals remained in this all-female group. On day 18, two males were placed in separate cages (Macrolon-II-cages, 18 x 24 x 14 cm) in the middle of each enclosure for a period of another 15 days, day 19 – 33. The bedding of the males was mixed once per week and the positions of the two cages were interchanged several times. The introduction of caged males was intended to signal mating opportunities to the females without enabling them to mate.

We collected data on the females' nest-box use for all 22 groups. Using this same data-set, fitness consequences of female social partner choice have been analysed in a previous publication (Weidt et al. in press). For ten groups, we carried out behavioural observations and sampled faeces for endocrine analysis at regular intervals before and after introduction of the males. To detect increased aggression, we additionally checked the females for scars and wounds at least once a week. In two groups one female each had to be removed before male introduction due to wounds inflicted by her group mates. Both animals recovered and wounds healed within a few days without additional treatment. In one trial, a single female escaped from the enclosure after male introduction. We proceeded with the five remaining females in these groups.

Behavioural Observations

For ten groups, behavioural observations were carried out 24 times, 12 times each before and after introduction of males with at the most one observation unit per day, beginning at day one. Observations took place during the females' activity period in the twilight and night phase between 17:30 and 22:00 h, enabled by red light in the experimental room. We recorded the frequency of agonistic interactions between females during a 1-hour period. The behavioural elements 'chase/flight', 'bite', 'attack', 'approach/retreat' and 'fight' were used according to Mackintosh (1981), Rusu and Krackow (2004) and Rusu et al. (2004). Furthermore, as an additional agonistic element, we included 'expel from nest-box', i.e. one female displaced another one from a nest-box.

We additionally recorded the number of nest-box changes for each female as a measure for activity. For two replicates, four and 17 of the 24 1-hour observation units were excluded from analysis as none of the females appeared outside a nest-box and no behavioural data were collected. For comparisons of the periods prior to and after male introduction, data were pooled for days 1-18 and 19-33.

Determination of social ranks

We used Elo-rating according to Albers and De Vries (2001) to describe social hierarchies among females of one replicate group. The method of Elo-rating provides sequential estimation of individual dominance strength based on the actual sequence of agonistic interactions (for detailed information see Albers & De Vries 2001). To calculate Elo-rating values we included all agonistic interactions within the 1-hour observation units, either resulting in a winner and loser, or undecided.

Based on the Elo-rating values, an estimated rank order can be derived at any moment in time. However, estimated ranks are only meaningful, if an assigned rank order is not altered by single interactions but is rather stable over time. We therefore carried out simulations (using the number of observed agonistic interactions per group) to obtain 95% confidence intervals of Elo-rating values reached by chance (when females would interact randomly). We only assigned females as 'dominant' or 'subordinate', when Elo-rating values were above or below this confidence interval, respectively. All females with values within the confidence interval were assigned as 'medium'.

The number of observed agonistic interactions over the course of the experiment varied between groups (9 – 455). We therefore calculated the confidence intervals for each group separately. All simulations were run with 100 repeats (the values did not differ substantially if running 100 or 1000 repeats), with 6 females per group and with a starting value of 1000, applying the rules of the Elo-rating method according to Albers and De Vries (2001). For each interaction, two individuals were drawn at random, and winner/loser were assigned based on these individuals' current Elo-rating and a uniformly distributed random number. Minimum and maximum values for the confidence intervals levelled off after approximately ten interactions, suggesting that meaningful results can be obtained when ten or more interactions have taken place.

We assessed the hierarchical structure of each group by using the final Elo-rating values at day 33 of the experiment as an individual's characteristic for its social rank. On the basis of the simulation results, we assigned each female as 'dominant', 'medium' or 'subordinate'. Nine groups showed at least ten agonistic interactions each, and one group nine agonistic interactions over the course of the experiment. We included all ten groups in the analysis.

To investigate whether male introduction had an impact on the hierarchical structure of female group members, we carried out a linear mixed-effects model (Pinheiro & Bates 2000) fitted by residual maximum likelihood with individual Elo-rating values at day 33 as the response variable, and individual Elo-rating values at day 18 before male introduction as the explanatory variable. Group identity was incorporated as a random term to account for effects due to same group origin. We conducted this analysis with eight groups, as two groups only showed one, respectively five agonistic interactions in the first part of the experiment and the resulting Elo-rating values were not considered meaningful.

Group activity

To determine the impact of male presence on female activity, we compared for each of the ten groups analyzed for dominance hierarchies the mean frequency of nest-box changes per observation hour between the time periods before and after male introduction. This was done using a Wilcoxon Signed Ranks Test.

Agonistic interactions

Similarly, to investigate the impact of male presence on the frequency of socio-negative behaviour among females, we compared for each group the mean number of agonistic interactions per observation hour between the time periods before and after male introduction with a Wilcoxon Signed Ranks Test.

Nest-box Use

For all 22 groups we collected daily data on the females' nest-box use on 30 consecutive days, between day 4 - 33. The location of each female was registered with a portable transponder reader (LID 500 Hand-Held Reader, TROVAN electronic identification systems) once a day at midday.

Nest-box use, specifically spatial association, was used as a measure for social partner preference (see also Weidt et al. in press), a relation suggested in several previous studies on house mice (Dobson et al. 2000; Hayes 2000; Manning et al. 1995; Rusu et al. 2004; Wilkinson & Baker 1988). We determined spatial associations according to the symmetrical index of Fager (I_{ij}-index) as modified by Kerth and König (1999). We calculated the expected probability that two females of a dyad meet in any of the nest-boxes by chance, and compared this expected value with the observed data using a binomial test. Two females were regarded as 'preferred partners', when they showed a significant positive association, meaning that they shared nest-boxes significantly more often than expected by chance. Females were regarded as 'non-preferred partners' when they shared nest-boxes in the range of random expectation. Comparisons between the periods prior to and after male introduction were based on data of nest-box use on day 4-18 and day 19-33, respectively.

Frequency of significant positive associations

To determine whether the presence of males altered the frequency of significant positive associations, we compared the proportion of 'preferred partner' dyads per group between the periods prior to and after male introduction with a Wilcoxon Signed Ranks Test. The proportion values can range from zero to one, with a value of zero indicating that no significant associations occurred, and a value of one that all dyadic associations of a group were higher than expected by chance.

Social partner preferences

To investigate the impact of male presence on female social partner preference, we tested whether the category of female association ('preferred' or 'non-preferred') in the absence of males was the same as in the presence of males. For this analysis we have chosen the two extremes in each group: the two females with the highest (significant) association and the two females with the lowest (non-significant) association. If more than one dyad in a group had the same highest or lowest association values, we randomly selected one. In two groups, all female dyads showed higher than random associations, resulting in only the 'preferred' pair to be used for analysis. In one group, one female in the lowest associated pair proved to have crippled genitalia and inner sexual organs at the end of the experiment, and the pair was excluded from analysis. We conducted a generalized linear mixed-effects model using a binomial error structure and the logit link function. The model was fitted with penalized quasi likelihood estimations (for details see Venables & Ripley 2002). We used the preference-category after male introduction as the binary response variable, the preference-category prior to male introduction as the fixed factor and group identity as a random term.

Monitoring Stress Hormones

For ten groups, we analyzed corticosterone metabolites (CM) in faecal samples according to Touma et al. (2003; 2004). In faecal samples, circulating hormone levels are integrated over a certain period of time and are little affected by single drastic events and episodic fluctuations of hormone secretion (Touma et al. 2004; Touma & Palme 2005), thus allowing us to assess longer-term endocrine profiles.

Faecal samples were taken at 6 defined dates (day 1, 4, 11, 18, 25 and 33) from each individual female. Samples on day 1 were taken prior to the release of the females into the enclosures, which we therefore defined as baseline levels of CM concentrations. As most social interactions and possible stress-responses were expected to occur during the first days of encounter, we collected the second sample on day 4, followed by weekly intervals. We always sampled the females between 07:30 and 09:00 h in the morning, avoiding possible fluctuations in the steroid excretion due to the circadian activity pattern (Touma et al. 2004; Touma & Palme 2005).

To obtain faecal samples from individual females, all females of one trial were removed from the enclosure between 07:30 and 08:00 h and singly placed in Macrolon-II cages (18 x 24 x14 cm), equipped with fresh bedding. After a period of 60 minutes females were released back into their enclosure. Faecal pellets were immediately collected from the cages and were frozen at -20℃. Possible endocrine responses due to the sampling event could not have influenced the current or following sample, as elevated CM concentrations in reaction to stressful events are only traceable in the faeces with a delay of 4-10 hours, depending on the time of day and the activity rhythm of the animals (for detailed information see Touma et al. 2003).

Faecal steroid metabolites were extracted according to the method described by Palme and Möstl (1997) and adapted for mice by Touma et al. (2003). Briefly, frozen samples were dried for two hours at 80℃. Each sample was homogenized and shaken with 20 µl of 80% methanol per mg faeces for 30 min on a multi-vortex. The ideal amount of faeces for further processing was 50 mg, the minimal amount used was 20 mg. After centrifugation (10 min at 2500 g), an aliquot of 500 µl of the supernatant containing steroid metabolites was frozen at -20℃ until analysis. To determine the amount of faecal CM, we used a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay, a method specifically established and validated for mice by Touma et al. (2003; 2004).

To investigate the effect of grouping females with unrelated, unfamiliar same-sex conspecifics on CM concentrations, we again carried out a linear mixed-effects model fitted with residual maximum likelihood. CM concentration was the response variable, the sampling bout (baseline level / day 1 or day 4) was used as a fixed factor and the individual was nested in group identity as a random term to account for potential similarities of individual females originating from the same enclosure.

We additionally carried out a linear mixed-effects model fitted with residual maximum likelihood to investigate potential factors altering CM concentrations. The difference in CM concentrations between the sampling bouts was the response variable, and we used male presence as a fixed factor. We additionally included mean number of agonistic interactions per group, individual activity and the final individual Elo-rating values as fixed factors as they may also affect CM concentrations. Furthermore, we included time, which refers to sampling bouts following changes in the experiment (day four, 11 and 18 before male introduction and day 25 and 33 after male introduction), as an ordered fixed factor and the interaction between male presence and the individual Elo-Rating values. In a stepwise backwards approach we removed the interaction from the model to investigate the main effects. Male presence nested in individual, which was again nested in trial identity, was used as a random term to account for potential similarities of females originating from the same enclosure in the absence and presence of males. We based this analysis on type three sum of squares to investigate each term independently.

To correlate behaviour with hormone responses, we matched the time frames of faecal sampling and respective observations. We used behavioural data taken during the two preceding days (this means approximately between 10 - 38 hours) prior to each faecal sampling event. Depending on the observation schedule, this time period might have included one or two observation bouts. For the analyses, we used the mean number of agonistic interactions occurring in each group per hour observation and the mean number of nest-box changes per individual as a measure of individual activity per hour observation relating to the faecal sampling events on day four, 11, 18, 25 and 33.

Statistics

Statistical models were carried out using R for Windows, Version 2.2.0 (R Development Core Team 2005) and the packages 'nlme' (Pinheiro et al. 2004) and 'MASS' (Venables & Ripley 2002). The model assumptions of normality and homogeneity of variances were verified graphically and were always met. Nonparametric statistics were conducted using SPSS 13.0 (SPSS Inc. Chicago, IL, USA). All tests were two-tailed and effects were regarded as significant at $p \leq 0.05$.

RESULTS

Social Hierarchies

In only four of the ten groups, one 'dominant' female each occurred, and none of the groups contained a female which was assigned as 'subordinate'. Most females were classified as 'medium' and showed rather similar Elo-rating values. Furthermore, social hierarchies were stable, independent of male presence. The final Elo-rating values at day 33 could be predicted by the Elo-rating values at day 18, prior to male introduction ($F_{1,39} = 399.46$, $p < 0.001$, Fig. 1). All four females, which were assigned as 'dominant' at the end of the experiment already held this classification at day 18, before males were introduced.

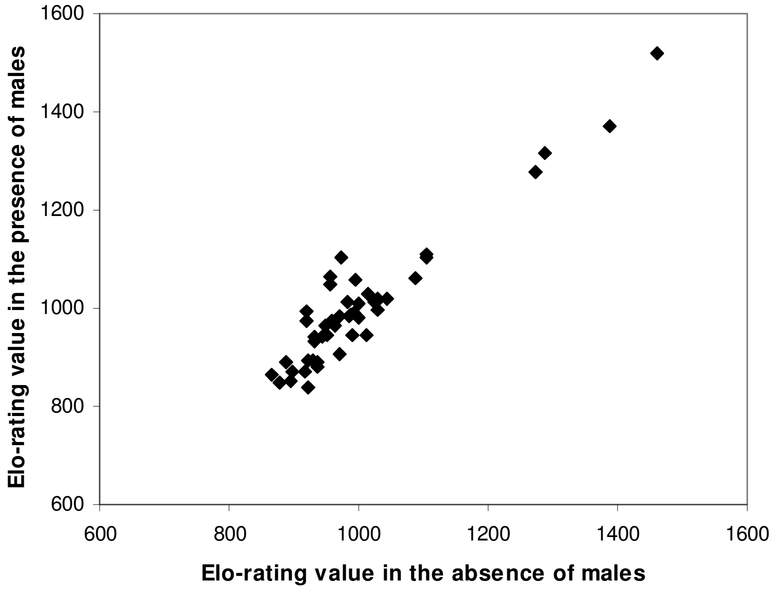


Figure 1 Relation between individual Elo-rating values at day 18, in the absence of males, and day 33, in the presence of males.

Group Activity and Agonistic Interactions

Group activity, measured as the mean frequency of nest-box changes per observation hour, increased significantly in the presence of males (Wilcoxon Signed Ranks test: Z = -2.70, p = 0.007; n = 10, Fig. 2a). In contrast, we found no significant difference in the mean frequency of agonistic interactions per observation hour between the time periods without and with males (Wilcoxon Signed Ranks test: Z = -1.58, p = 0.11; n = 10, Fig. 2b).

Significant Associations and Partner Preferences

The proportion of 'preferred partner' pairs did not differ significantly between the time periods prior to and after male introduction (Wilcoxon Signed Ranks test: Z = -0.67, p = 0.51; n = 22, Fig. 2c). Within groups, 7-100% of the pairwise associations among females were significantly higher than expected by chance in both periods.

However, even though the overall ratio of significant associations did not change significantly, 16 of the 41 dyads showed a change in their preference category ('preferred' versus 'non-preferred') in the presence of males. The preference category of the highest and lowest associated pairs after male introduction could thus not be predicted by the preference category of those dyads in the absence of males ($F_{1,18}$ = 2.2, p = 0.15). Changes of the preference category occurred in both directions. Five out of 19 dyads which were classified as 'non-preferred' pairs in the presence of males were classified as 'preferred' prior to male introduction, and 11 out of 22 dyads classified as 'preferred' pairs after male introduction were classified as 'non-preferred' in the absence of males.

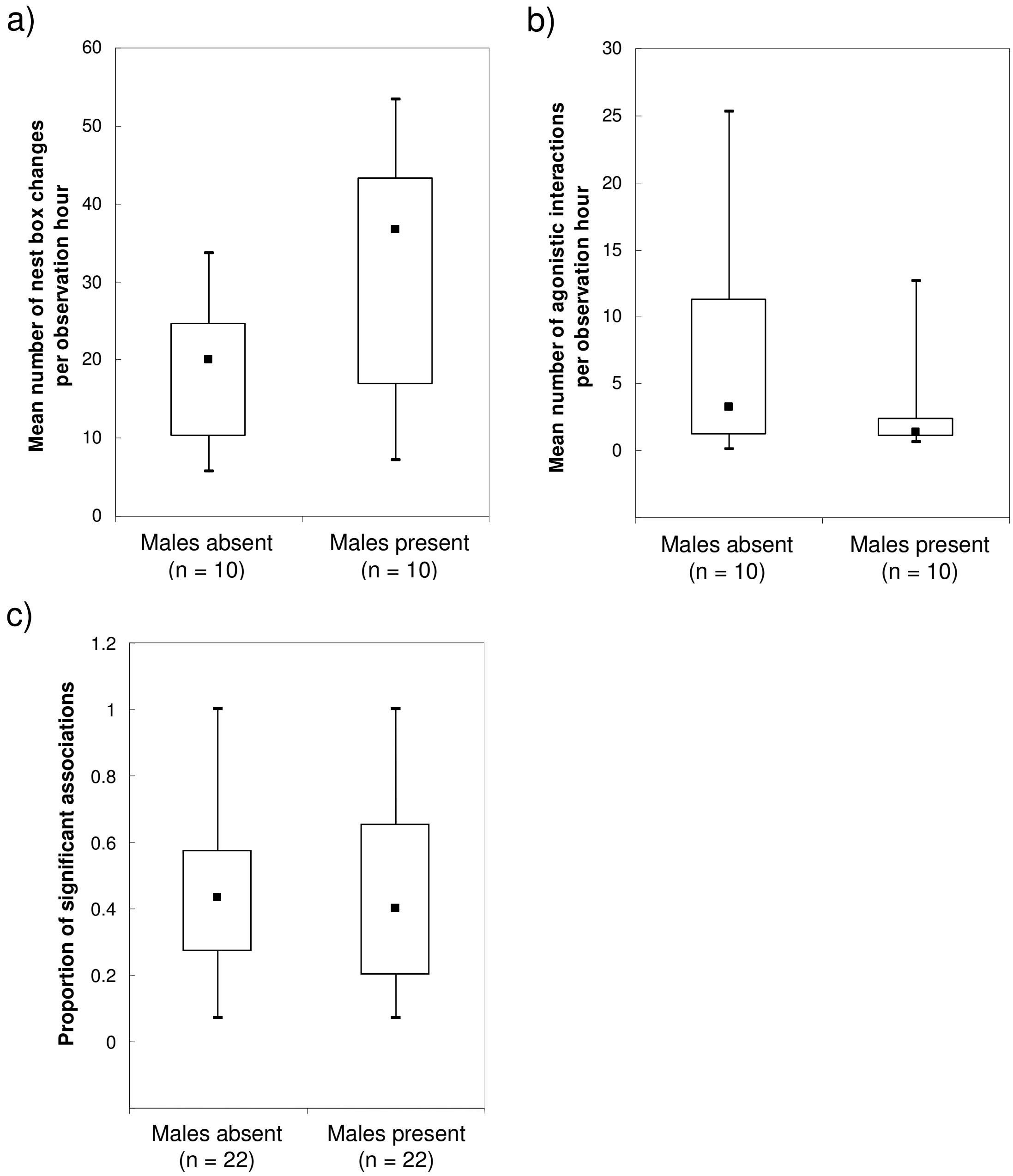


Figure 2 Influence of male presence on a) mean number of nest-box changes, b) mean number of agonistic interactions and c) proportion of significant associations. Data are shown as medians, box: interquartile range 25%-75%, whiskers: Min.-Max..

Stress Hormone Production

The grouping of unrelated, unfamiliar females in a rather large enclosure with several nest-boxes, feeding and drinking sites did not induce elevated stress hormone production lasting for the first three days. The CM concentrations on day four of the experiment did not differ significantly from the baseline levels collected when females were still housed with same-sex litter mates ($F_{1,26} = 0.17$, $p = 0.688$; Fig. 3).

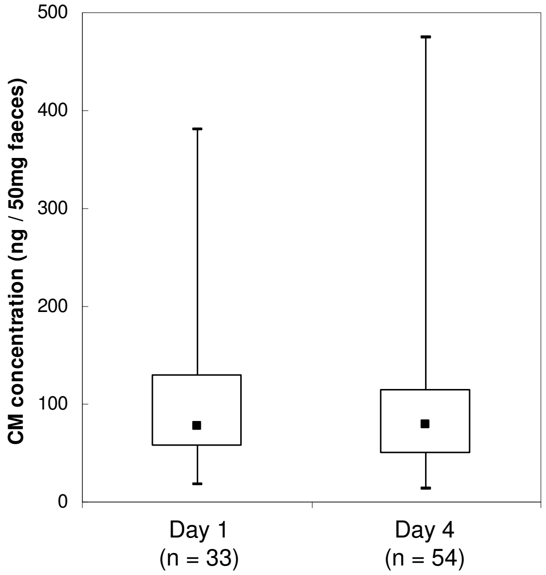


Figure 3 Concentrations of corticosterone metabolites (CM) in the faeces at day 1 (baseline) and day 4 of the experiment. Data are shown as medians, box: interquartile range 25%-75%, whiskers: Min.-Max.. Sample size differs between day 1 and day 4 as fewer individual faecal samples could be collected during the first than during the second sampling bout.

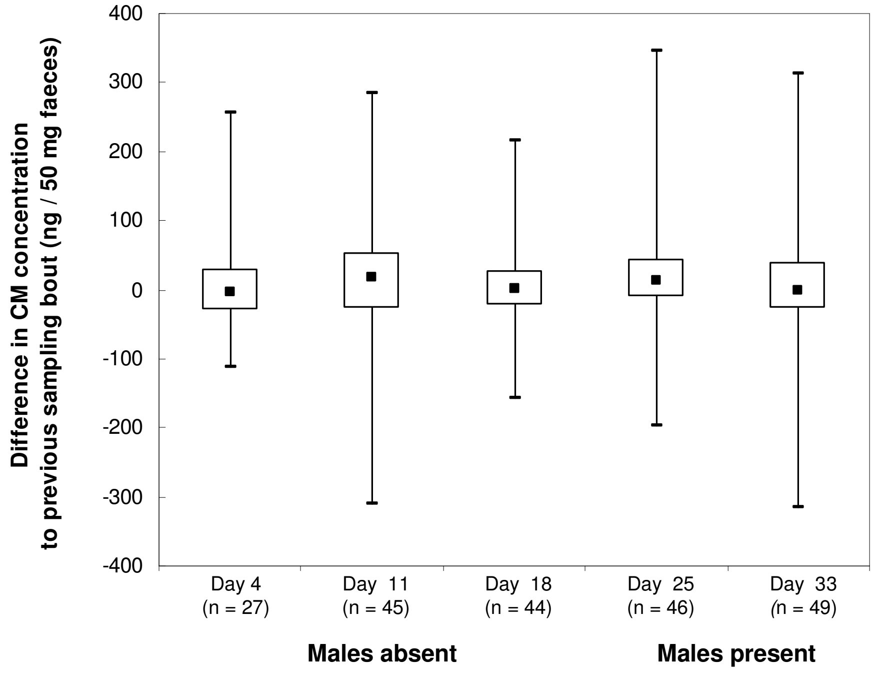


Figure 4 Changes in concentrations of faecal corticosterone metabolites (CM) in relation to the previous sampling bout before (day 4, 11, 18) and after male introduction (day 25, 33). Data are shown as medians, box: interquartile range 25%-75%, whiskers: Min.-Max.. Sample sizes for each sampling bout refer to the data analyzed in the model.

We found no significant effect of the mean number of agonistic interactions per group ($F_{1,106}$ = 0.37, p = 0.55) and of individual activity ($F_{1,106}$ = 0.03, p = 0.86) on stress hormone production. In addition, time had no systematic effect on CM concentrations ($F_{2,106}$ = 0.13, p = 0.88; Fig. 4) and the interaction between the presence of males and the individuals' final Elo-rating values was not significant ($F_{1,45}$ = 0.31, p = 0.58). There was also no main effect of the final Elo-rating value ($F_{1,44}$ = 0.23, p = 0.64) and of male presence ($F_{1,46}$ = 0.28, p = 0.60; Fig. 4) on stress hormone production after removing the interaction from the model.

DISCUSSION

The introduction of caged males, which allowed olfactory, acoustic and limited physical contact (but no mating) did not induce competition in group living female house mice. Male presence, nevertheless, significantly altered the females' preference for social partners.

Mate Competition among Female Group Members

Intrasexual aggression

Agonistic interactions among females were generally low and did not increase after male introduction, even though overall group activity rose so that females were expected to meet and interact more frequently. Female-female mate competition in polygynous and monogamous mammals is indicated by intrasexual aggression when breeding opportunities occur, as has been shown for example in red deer (Bebie & McElligott 2006), Mongolian gerbils (Scheibler et al. 2004) and house mice (Rusu & Krackow 2004; Palanza et al. 2005). Presence of or olfactory cues from unfamiliar males elicit oestrous cycles in female house mice and signal the opportunity to reproduce (Marsden & Bronson 1964; Bronson & Whitten 1968). In our study, male presence did not cause increased female-female aggression, suggesting that females did not compete over access to mating partners.

Egalitarian female relationships

The assumption that males did not induce competition is further supported by our findings on the hierarchical structure among female group members. Only in the minority of groups (40%), one female was assigned as dominant, and subordinates occurred in none of the groups. Most females were classified as medium. Thus, we found no pronounced hierarchical structure among female group members, and this situation remained unchanged when males were introduced (see Fig. 1).

Social dominance is a feature of many animal societies (reviewed in Gauthreaux 1978; Piper 1997), where high social rank is typically associated with priority of access to resources, such as food, nest-sites or mates (Wilson 1975; Appleby 1980; Brodsky et al. 1988; Stahl et al. 2001). Dominance hierarchies are considered as a means to reduce direct and indirect costs of competition (Archer 1988; Piper 1997). Females are therefore expected to develop social hierarchies whenever group members compete, as for example over mating partners. When within-group competition is low or absent, however, females are thought to have rather egalitarian relationships (Sterck et al. 1997). The latter situation applies to our study, where distinct hierarchies among female group members are lacking both in the absence and in the presence of males.

Group life and corticosterone profiles

We also found no indication for increased competition in the presence of males on the physiological level. The introduction of males had no effect on the concentration of corticosterone metabolites (CM) measured in the females' faeces. In general, social interactions can have a profound impact on endocrine parameters in mammals (e.g. Henry & Stephens 1977; Svare 1983), as for example through the release of glucocorticoids, or 'stress hormones', which are emitted into the bloodstream in response to activation of the hypothalamus-pituitary-adrenal axis (Wingfield & Ramenofsky 1999; Sapolsky et al. 2000; Sapolsky 2002). In reptiles, birds and many rodents the primary glucocorticoid is corticosterone (Sapolsky 1987; Schwabl 1995; Wingfield & Ramenofsky 1999; Creel 2001). Individuals which undergo disruption in social rank, involvement in agonistic encounters or which exhibit intrasexual conflicts with group members (such as competition for mating partners), frequently show elevated corticosterone levels (Sapolsky 1987; Schwabl 1995; von Holst 1998; Wingfield & Ramenofsky 1999; Creel 2001; Goymann & Wingfield 2004). Increased stress levels may indeed be directly linked to reproductive competition. Glucocorticoid concentrations substantially increased during the mating season in wolves (Sands & Creel 2004), or in Mongolian gerbils when founder females were replaced and competition for reproduction was elicited (Scheibler et al. 2004). We therefore expected that mate competition among female group members, if existent, should be traceable on the physiological level by an increase in faecal CM. Introduction of males, however, had no influence on female CM concentrations.

Still, effects of male presence on female CM concentrations might have been 'masked' by rank related differences among females. Apart from the fact that females may exhibit different baseline glucocorticoid levels depending on their social ranking (for details see McEwen & Wingfield 2003; Goymann & Wingfield 2004), females of different social status may also react differently on imposed stressors, which may prevent the detection of a general reaction pattern. Such individual stress reactions were demonstrated in Mongolian gerbils for periods of high aggression (Scheibler et al. 2004). Sands & Creel (2004), on the other hand, showed in their study on free-living wolves that glucocorticoid levels significantly rose during the mating season for all group members, independent of their social ranking. In our study, we neither found a general rank effect, nor, more importantly, a differential effect of male presence on CM levels of females with different social rankings (there was no significant interaction between male presence and the individuals' Elo-rating values). Given the relatively egalitarian social structure among females, that lack a pronounced dominance hierarchy, this result is not very surprising.

In addition to the missing impact of male introduction on stress levels, group life among female house mice generally appeared to be free of lasting and drastic stressors traceable in faecal CM concentrations, at least when resources such as nesting sites, food and water were not limiting, as in our study. CM differences did not vary considerably over time and were not higher between day 1 and day 4, after females were removed from their home cages, where they were housed with same-sex litter mates, and grouped with unfamiliar, unrelated females. Our results are in line with previous studies by Brown & Grunberg (1995), demonstrating that stress levels in female rats and mice, in contrast to those of males, are not strongly affected when housed in groups, even under crowded conditions. Behavioural strategies in coping with social and environmental challenges differ in male and female polygynous mammals, given their different social and reproductive roles. For social females, therefore, group life should generally not impose severe stress, which may have fatal consequences when chronic (Sapolsky 2002; Sapolsky et al. 2000). Nevertheless, it is surprising that this is even the case in groups of previously unfamiliar and unrelated females, especially as females in natural house mouse groups are generally close kin (Petras 1967; Lidicker 1976; Pennycuik et al. 1986). However, the ability to behaviourally and physiologically cope with strangers might yet be an important characteristic, as females occasionally emigrate from their natal territories and either integrate into another group or establish a new one (Baker 1981; Gerlach 1990, 1996).

Absence of female competition over males

Overall, we found no behavioural or physiological indication that male presence elicited competition among female group members, suggesting that females are not constrained in access to males. This contrasts with Rusu & Krackow (2004), who described elevated female aggression and the existence of dominance relationships in groups of three females living with one male in similar sized enclosures. The authors concluded that short oestrous cycles and long copulation bouts constraint access to the mating partner when females are reproductively synchronized. The discrepancy to our own data might be explained by two arguments. First, in groups of three, females may not compete over males but over a social partner (two of the females compete over access to the third female). In larger groups, such constellations may be rare. Second, competition over males is mainly expressed in the presence of a single male (see also Rusu & Krackow 2004). Cues from several males, however, signal unlimited access to males, given that reproducing females are free to move between male territories, as suggested by the occurrence of multiple paternity in house mice (Dean et al. 2006).

The absence of female competition, on the other hand, is in line with recent findings of Palanza and colleagues (2005) stating that female intrasexual competition is regulated by the timing of female-female settlement in relation to male settlement. This conclusion is supported by game theory models (Maynard Smith 1973, 1982; Maynard Smith & Parker 1976), which suggest that prior social experiences and possession of a resource influence the intensity and outcome of competitive interactions. Females that interacted at the same time or prior to cohabitation with a male (symmetric contest, females were equal in terms of prior residence and association with male), as in our study, showed little aggression and a high degree of reciprocal tolerance (Palanza et al. 2005). Females that first cohabitated with a male for some days before other females were introduced (asymmetric contest, females were not equal in terms of possession of a resource), however, were highly aggressive and intolerant (Palanza et al. 1994; 2005). The social organization of female house mice thus appears to be more complex and variable than the clear-cut territorial dominance observed among males (Palanza et al. 2005).

Social Partner Preferences

Female choice of a social partner is reflected in preferential cohabitation (significant spatial associations) in house mice (Weidt et al. in press). The proportion of such positive associations between female group members did not differ in the absence and presence of males, indicating that male introduction did not influence the females' general sociability or choosiness concerning the number of social partners. Male presence, however, significantly altered female preferences. Overall, 16 of 41 female dyads revealed modified preferences in the presence of males. Partners, that were preferred in the absence of males were no longer chosen when males were present, or vice versa. Given that social partner choice in the presence of males yields significant fitness benefits (Weidt et al. in press), our data allow to conclude that partners for cooperative reproduction are only chosen when reproductive opportunities are imminent, that is in the presence of males. According to Dugatkin and Sih (1995), individuals may display different partner preferences in different social contexts. Females may for example use different decision rules when choosing between potential mates or when choosing partners for antipredator behaviour or thermoregulatory benefits. In our experiment, the necessity to choose partners for cooperative breeding activities only occurs when mating and breeding opportunities are at hand. Prior to male introduction, nesting partners are seemingly chosen according to other cues.

The observed change in partner preferences may, on the mechanistic level, be explained by alterations of the females' reproductive state. As mentioned before, female reproductive cycles are induced by male stimuli, which is associated with drastic changes in the females' hormonal system. Ovarian hormone fluctuations in turn can lead to behavioural changes, for example related to emotionality or anxiety (Gray & Levine 1964; Mora et al. 1996). As a consequence, a female's reproductive state may generally modulate social relationships among females (Palanza et al. 2005). This may also result in alterations of partner preferences during different reproductive phases, as shown for example in women (Puts 2005) and toads (Lea et al. 2000). However, other social factors and cues such as pheromones may also be potential modulators of female social relationships.

In conclusion, our results showed that male presence indeed influences the structuring of female societies, however not by eliciting competition among females, but by altering female preferences for social partners. Males make females change their mind. This finding emphasizes the necessity of considering male presence in studies on female social partner choice in the context of reproductive cooperation. Further studies are needed to clarify the mechanisms of male impact on female social partner decisions.

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Females have a choice: Communal nursing decisions in free-ranging wild house mice (*Mus domesticus*)

ANDREA WEIDT & BARBARA KÖNIG

Institute of Zoology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Communal nursing, where females share milk between own pups and young produced by another mother, is a peculiar case of cooperative care. As lactation is energetically costly, a female's investment in alien young is remarkably high. House mice (*Mus domesticus*) are one of the few species which are known to exhibit indiscriminant communal nursing behaviour. Lab experiments suggest that communal nursing is adaptive in house mice and that it provides direct fitness benefits. However, this reproductive cooperation assumes that both partners successfully reproduce and wean pups. In order to establish such an egalitarian relationship, the choice of an appropriate partner is crucial. Indeed, females display non-random preferences for cooperation partners and partner choice yielded significant fitness benefits under laboratory conditions. Yet, the question remains to what extend females can express social partner choice and consequently communal nursing decisions under natural conditions, where ecological and social constraints of group life are at work. Preliminary evidence on house mice in semi-natural enclosures suggest that social partner choice is indeed constrained by effects of group living. Communal nursing appeared to be obligatory and was suggested to be an unavoidable by-product of sharing nesting sites. We investigated the availability and use of options to nurse communally as well as spatial associations between potential nursing partners in a population of free-ranging wild house mice to assess if a female's choice is really constrained by sociality. If confinement would be strong, we expect that females use each option to nurse communally and that they always choose closely associated nesting partners. However, our data revealed that females can be choosy in a free-ranging population. Female house mice did not use each option to nurse communally and communal nest sharing did not inevitably lead to communal nursing activities if this was an available option. Interestingly, also population density did not influence a female's probability to use a communal nursing option. The use of options though was significantly affected by the number of potential nursing partners, suggesting that the availability of appropriate cooperation partners is indeed crucial and strongly determines communal nursing decisions.

Keywords

Associations, communal nursing, cooperation, female decisions, house mice, *Mus domesticus*, options, partner choice, sociality, social partners

INTRODUCTION

Communal nursing, where milk is shared between own pups and young produced by another mother, is a peculiar phenomenon in female mammals. It occurs in all major mammalian taxa and seems to have evolved independently under a variety of conditions (reviewed in Packer et al. 1992). The development of cooperative relationships is, according to Emlen & Oring (1977), a secondary effect of group living and not the primary basis of sociality. Nevertheless, cooperative activities may lead to an increase in fitness benefits and can make group live additionally profitable (West-Eberhard 1979). However, seemingly cooperative behaviour may also have developed as a non-beneficial or even costly side effect of group living (e.g. Pusey & Packer 1994), but will be maintained as long as the benefits of group living outweigh the fitness costs (Alexander 1974). Accordingly, several hypotheses, including adaptive and non-adaptive explanations, have been raised to explain the phenomenon of sharing milk with alien young (reviewed in Lewis & Pusey 1997; Hayes 2000; Roulin 2002; König 2006). Non-adaptive explanations include for example milk theft by parasitic young (McCracken 1984; Murphey et al. 1995) or misdirected maternal care (e.g. Fogden 1971; Maniscalco et al. 2007). Adaptive explanations of communal nursing are indirect fitness benefits by kin selection (e.g. Eberle & Kappeler 2006) or direct fitness benefits due to increased reproductive success and improved survival of own offspring (e.g. Sayler & Salmon 1971; Mennella et al. 1990; König 1994b).

Communal nursing behaviour is common among rodents (reviewed in Packer et al. 1992; Hayes 2000), including house mice (Sayler & Salmon 1969, 1971; Wilkinson & Baker 1988; König 1994a; Manning et al. 1995; Dobson et al. 2000). House mice (*Mus domesticus*) are special in that they display an extreme case of communal nursing behaviour: they are one of the few species where own and alien pups are nursed indiscriminately when pooled in one nest, even under restricted feeding conditions (König 1989). Especially as the females' investment in alien young is thus remarkably high, female house mice offer an interesting system to study communal nursing decisions.

Wild house mice live in groups that are typically characterised by one territorial male, few, if any, subordinate males and several breeding and non-breeding females (Reimer & Petras 1967; Selander 1970; Lidicker 1976; Mackintosh 1981; Anderson & Hill 1965). Females often remain in their natal territory, but occasionally disperse and successfully immigrate into other breeding units, where they encounter unrelated and unfamiliar same-sex conspecifics (Anderson & Hill 1965; Bronson 1979; Baker 1981).

Laboratory experiments suggested that communal nursing in wild house mice, which assumes that two reproducing females successfully reproduce and wean pups, provides direct benefits for both partners (for a recent review see König 2006). To achieve such an egalitarian relationship of two successfully reproducing females, and thus benefit from cooperation, choosing the right social partner was shown to be crucial. Under laboratory conditions, females displayed preferences for specific cooperation partners and such social partner choice yielded significant fitness benefits (Weidt et al. in press). However, the question remains to what extent females express social partner choice and consequently communal nursing decisions under natural conditions, where ecological and social constraints of group life are at work. Such constraints may for example include confinement of space and resources, close spatial proximity to other group members, including the sharing of nesting sites, as well as potential competition for mates and social partners. Indeed, in a study of Manning et al. (1992), carried out under semi-natural conditions, social partner choice seemed to be strongly confined and solitary nests mainly occurred when there was no opportunity to nest communally. Communal nursing appeared to be an obligatory behaviour and it was suggested that it may be an unavoidable by-product of sharing same nesting sites (Manning et al. 1995; Hayes 2000).

To investigate if the expression of social partner choice apparent under laboratory conditions is indeed strongly confined in natural house mouse populations, we investigated the availability and usage of communal nursing options as well as the spatial associations among reproducing females on a population of free-ranging wild house mice. If confinement would be strong we expect 1) that females always use the option to nurse communally, meaning that single nests only occur if females have no option to nurse communally and 2) that females, which share nesting sites during non-reproductive periods will inevitably nurse communally when being reproductive.

METHODS

Study Site & Study Population

In 2002 we established a population of free-ranging wild house mice (*Mus domesticus*) in a barn in Illnau, a village 20 km outside of Zurich, Switzerland. We started our study population with four adult male and eight adult female house mice. Founder individuals originated from two different capture sites (two males and four females each), two farm houses situated within a 5 km range of the barn. Between June and October 2002, founder animals were caught with life-traps (Landi, Switzerland) baited with peanut-butter and oat flakes. Prior to the simultaneous release in the barn, all individuals were equipped with trovan® transponders, subcutaneously injected pit-tags for individual identification (ID 100: 0.1 g weight, 11.5 mm length, 2.1 mm diameter; TROVAN electronic identification system). For genetic analysis, we additionally took tissue samples (ear punches) of all individuals.

The study barn was 72 m^2 in size and consisted of a concrete floor, covered with saw-dust and hay. In November 2002, the barn area was divided into four separate quarters by 75 cm high aluminium plates, where one male and two females each (originating from the same capture site) were introduced. The ground area was structured with branches, plastic tubes, bricks, some hideouts and numerous PVC barriers (1-2 m length and 20-30 cm height). After successful reproduction of the founder individuals, we opened pre-prepared passages between all quarters in August 2003. Already at the outset, forty nest boxes (PVC, 15 cm diameter, 15 cm height, 2 entrance/exit tubes) were distributed evenly in the whole area and food (grains and oat flakes as well as food pellets (laboratory animal diet for mice and rats, no. 3804 & 3336, Provimi Kliba SA, Kaiseraugst, Switzerland)) and water was given ad libitum at eight feeding and 7-8 drinking sites (see Figure 1). The walls which were composed of bricks in the lower part and wooden boards in the upper part additionally enabled the mice to climb, thus offering a three dimensional structuring. Four PVC tubes (4.5 cm diameter, 40 cm length) fixed in the walls near the ground allowed mice to move freely in and out of the barn from August 2003 onwards. Due to a steep population decline inside the barn area, these tubes were closed in June 2004. However, from December 2004 onwards mice could leave and enter the barn again via one single flexuous 5 m PVC tube (4.5 cm diameter). Overall, this set-up represents conditions similar to those met by natural house mouse populations living at farm houses where animal food or grain is stored.

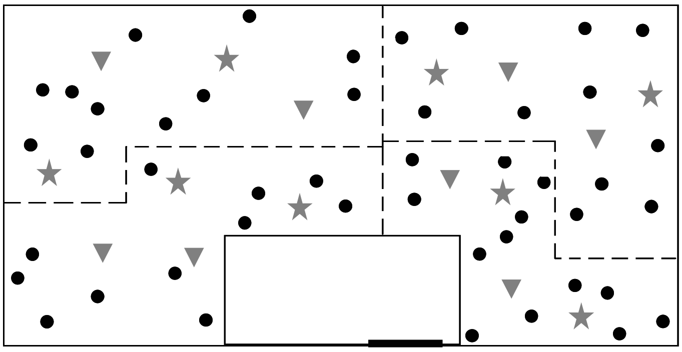

Figure 1 Barn set-up for the study period from November 2002 until December 2004: circles: nesting sites (N = 46), stars: feeding sites (N = 8), triangles: drinking sites (N = 8), dashed lines: 75 cm high aluminium walls with several passages structure the area in four main quarters, solid lines: outside walls and indication of separate area for handling and observing animals next to the entrance door (thick line).

Population Monitoring

Continuous monitoring of the population took place from November 2002 until December 2004. Generally, we examined the whole population inside the barn at weekly intervals during the animals' least active time, i.e. during daytime hours. For that purpose the entrance/exit tubes of all nest-boxes were plugged and each nest-box was successively investigated. All individuals in the nest-boxes were weighed, and the pups' age and sex was determined. Once they had reached an age of at least eight days tissue samples (ear punches) were taken for genetic analyses. Moreover, all individuals weighing at least 18 g were pit-tagged with trovan® transponders for persistent individual identification. They were simultaneously tissue-sampled for genetic analysis, potentially the second time. Furthermore, we assessed the reproductive status of all adult females by visually investigating the abdomen and the teats. All animals which did not stay inside nest-boxes during such population examinations were caught by life-traps or by hand and examined as described above. Dead individuals, including pups, were registered and removed from the barn. Tissue samples of the dead individuals were taken whenever their identification was not unequivocal. All tissue samples were stored in plastic tubes with 95 % ethanol until further processing. In addition to the weekly population examinations, the nesting pattern of all pit-tagged individuals was determined 3-5 times per week. All nest-boxes, hide-outs and tubes were non-invasively checked during the animals' resting period using a portable transponder-reader (LID 500 Hand-Held Reader, TROVAN electronic identification systems). As this study was part of a long-term project, a continuous data collection without interruptions could not be ensured throughout the whole time period. At times, population examinations and nesting pattern checks were carried out at lower frequency and consequently not all litters were sampled and not all adult individuals pit-tagged immediately, leaving some information gaps. Nevertheless, we attempted to obtain an optimum of information.

Litter assignments

Litters were assigned to their mother using a combination of observational and genetic data. We considered the females' changes in body-weight (increase before birth and distinct decrease at time of birth), the morphology of the abdomen and the teats (visual signs of embryos and of lactation, respectively) as well as the females' location in relation to the pups' location at the day of birth and after giving birth as observational data on potential maternity.

In addition, we carried out parentage analyses using 13 microsatellite loci (D11Mit150, D12Mit91, D13Mit88, D18Mit194, D2Mit145, D7Mit17, D9Mit201, D10Mit230, D14Mit44, D16Mit139, D4Mit227, D5Mit122, D6Mit390) characterized in the Mouse Genome Informatics database (Eppig et al. 2005) at www.informatics.jax.org. These loci did not deviate from Hardy-Weinberg equilibrium when tested on all adults living in the barn more than one generation after the founder event. Parentage analysis was performed using Cervus 3.0 by Tristin Marshall (Field Genetics Ltd.). We specified a unique set of candidate mothers and fathers for each litter. Candidate mothers and fathers were those adult females and males known to be alive at the approximate date of conception (considered to be 19 days before birth). We accepted parentage assignment when confidence was 95% or greater. If confidence in the parent pair was less than 95%, we nevertheless accepted the maternity assignment if its confidence was 95%. Genetic samples were not available or did not always amplify for all pups.

Communal Nests & Communal Nursing Options

Once pups of different litters are placed in one single nest, females nurse them indiscriminately, even under constricted feeding conditions (König 1989). It can thus be safely assumed that communal nursing takes place whenever different litters are combined in one nest. At day 17, the start of the weaning period, pups begin to consume solid food (König & Markl 1987) and nursing becomes less important. We therefore defined communal nests as nests containing two or more different litters, which are not yet being weaned (below the age of 17 days).

For a pregnant female shortly before giving birth, potential social partners for communal nursing activities (communal nursing options) are all females which have reproduced lately, maximally 16 days prior to the birth of her own litter, and which are spatially accessible and are thus known to the female. For litters which were born on the same day and the order of birth was unknown, we randomly assigned a birth order. The mother of the litter assigned as born first was then indicated as a potential nursing option for the mother of the litter assigned as born second.

We assumed spatial accessibility and knowledge about another female whenever females used the same spatial area. The spatial area an individual female uses is defined by the number and distribution of nesting sites, and is further referred to as a female's home range. For each possible dyad of reproducing females, we therefore recorded the number of nesting sites commonly used (however not necessarily on the same day) during the time period when both females were simultaneously present in the barn. Forty-six nesting sites, 40 nest boxes as well as six additional nests established by mice inside the barn area during the study period, were taken into account (see Figure 1). Whenever two females used at least one common nesting site in the same time-period, meaning that their home ranges overlapped, we assumed that those females had knowledge about and could access each other. This analysis could only be carried out for pit-tagged females where nesting pattern information was available.

However, apart from using nesting pattern data, the original spatial arrangement of the barn in four different quarters proved to offer a simple reliable cue for estimating home range overlap: all reproducing females giving birth in the same quarter always used at least one nesting site in common. These assignments of accessible females coincided strongly with the assignments using nesting pattern data, which always also included all reproducing females in the same quarter, but assigned some additional females of neighboring quarters as communal nursing options. We thus used the quarter, where a litter was born, as a conservative measure for assigning communal nursing options when mothers were not pit-tagged or unknown (which was the case for 15 out of 111 litters).

We investigated the use of options, i.e. if a female, who had at least one option to nurse communally, actually reared her litter in a communal nest or if she reared her litter solitarily without a nursing partner. Additionally, we were interested in the parameters affecting the decision to nurse communally or not. For all respective parameters described below, we therefore compared the cases where at least one option to nurse communally was used (‘litter reared communally’) with all cases where females did not use any of the options and nursed their litter solitarily (‘litter reared solitarily’).

Female Nesting Partners

To determine whether differences in the use of communal nursing options can be explained by a general variation in the females’ spatial behaviour towards same-sex conspecifics (i.e. if females, when nursing solitarily, generally tended to avoid other females already prior to reproduction), we compared the number of female nesting partners between mothers which established a communal nest and mothers which nursed solitarily despite available options, using a Mann-Whitney U-test. For each litter with at least one option to nurse communally, we determined the mean number of additional females sharing the nesting site with the mother prior to the birth of the litter, first, while the mother was still non-reproductive and, second, while the mother was already pregnant. We calculated the mean number of additional females sharing a nesting site for the non-reproductive and pregnant period using nesting pattern information from two sampling days each. For the period of the mothers’ pregnancy, we counted 8 days backwards from the litter’s birth date and used the two sampling days closest to that point in time. Similarly, for the non-reproductive period we used 28 days prior to the litter’s birth data as a reference time. Data were excluded for the non-reproductive or pregnant period when the mother was still nursing her previous litter. This analysis could only be carried out for pit-tagged females.

Individual Associations

We additionally investigated the overall individual associations of prospective mothers to their potential nursing partners during the mothers’ non-reproductive periods. Dyadic associations were calculated according to the symmetrical index of Fager (Iij-index, modified by Kerth & König 1999), using population monitoring data. The Fager’s index gives the ratio between the number of days where both females were simultaneously found at the same nesting site and the number of days where both females were present in the barn. Association indices were only calculated when females were simultaneously present on at least five sampling days. In addition, analysis was only possible for litters and potential options when mother assignments were resolved and when females were pit-tagged and thus spatial information available. For all litters reared communally, we calculated dyadic associations of the prospective mother to each of her chosen nursing partners. Those association values were compared with the association values of dyadic associations between prospective mothers, who nursed solitarily despite communal nursing options, to all their potential, but not-chosen nursing partners. We only included those sampling days where the prospective mother was non-reproductive, that is when she was neither pregnant (time period 20 days prior to birth) nor lactating (time-period until 21 days after giving birth). The reproductive state of the mothers' potential partner was not taken into account for this analysis. If, for subsequent litters, one female had the option for a specific nursing partner repeatedly and repeated her nursing decision, we included this dyad only once. However, if one female took a potential partner at one occasion and declined this same potential partner another time, we included the association of this dyad in both categories ('litter reared solitarily', 'litter reared communally').

Determinants of Communal Nursing Decisions

Finally, to determine whether the decision to nurse communally is affected by population density or by the actual number of available nursing options, we carried out a generalized linear model (GLM). To correct for potential overdispersion we used a quasibinomial error structure (Venables & Ripley 2002). The 'nursing decision' (litter reared solitarily/communally) was the binary response variable, 'population density' and 'number of available communal nursing options' were the explanatory variables. We calculated population density, given as the number of adult female and male mice per square meter, once per month based on population examination data. All individuals with at least 42 days of age or a weight of at least 16 g (when age was unknown) were counted as adults. For each litter we assigned the population density calculated for the corresponding month when the litter was born.

Statistics

Nonparametric statistics were conducted using SPSS 13.0 (SPSS Inc. Chicago, IL, USA). The statistical model was carried out using R for Windows, Version 2.2.0 (R Development Core Team 2005). All tests were two-tailed and effects were regarded as significant at $P \leq 0.05$.

RESULTS

Reproduction, Reproductive Skew & Communal Nests

Throughout the study period, we counted a total of 111 litters with 526 pups. 105 (95%) of those litters were assigned to 40 different mothers using observation and genetic data. Six litters could not be assigned. Genetic samples of 213 pups (40.5%), originating from 73 litters, were included for genetic parentage assignments. For 32 of the litters no genetic information was available and litters were assigned to the most likely mother due to observational data. Overall, reproduction was rather skewed within the population. Only 40 out of 195 females gave birth to a litter and even among the reproducing ones, 40% (42) of all litters were assigned to only seven mothers (including the founder females) with five to eight litters each. Sixty-nine (62%) out of the 111 litters grew up in single nests, whereas 42 (38%) were reared in a communal nest. Of those 42 litters, 28 participated in communal nests which consisted of two different litters, 10 litters participated in communal nests of three litters and four litters composed a single four-litter-nest.

Communal Nursing Options & Decisions

In 64 out of the 111 litters, the mother had at least one option to nurse communally, meaning that at least one other litter with an age of at maximum 16 days was present in the female's home range.

In only 24 (37.5%) of the 64 cases the option to establish a communal nest was actually used. In 10 (15.6%) cases, litters ended up in a communal nest with a later-born litter. Thirty (46.9%) of the 64 litters were reared solitarily even though their mother had the option to nurse communally. The number of communal nursing options for one single mother varied between one and six. A maximum of three options were taken at the same time, which means that pups were placed in a communal nest which already consisted of three different litters. Seven mothers showed both categories of nursing decisions when they had the option to nurse communally and established communal and solitary nests.

For all further analyses we compared the 24 cases where females used at least one option and litters were reared communally with the 30 cases where females did not use any of the options and litters were reared solitarily (Table 1). However, we excluded the 10 litters, where the mother did not use any of the options to nurse communally, but was later on chosen by another female as a nursing partner. As we cannot rule out that those mothers also participated in the decision to nurse communally, we omitted the cases from the analyses to avoid the risk of assigning them to the wrong category. Accounting for the fact that the data set was rather complex, we gave an overview over the data composition for each of the following analysis in Table 1.

Female Nesting Partners

Neither during the non-reproductive period (Mann-Whitney U-Test: $U = 17.0$, $P = 0.097$, Table 1 & Figure 2a) nor during pregnancy (Mann-Whitney U-Test: $U = 69.0$, $P = 0.61$, Table 1 & Figure 2b) did mothers, which nursed their litter solitarily and mothers which nursed their litter communally, differ significantly in their spatial behaviour towards other female group members. Prospective mothers, who had at least one option to nurse their following litter communally, but reared them solitarily, were as social prior to nursing as prospective mothers who reared their litter communally later on. We even found a weak trend towards a higher number of female nesting partners during the non-reproductive period for prospective mothers ending up in a single nest. The mean number of additional females sharing a nesting site with the prospective mothers varied between zero and 3.5.

CHAPTER 3

Females have a choice:

Communal nursing decisions in free-ranging wild house mice

(*Mus domesticus*)

to be submitted

Table 1 Overview over data composition for all analyses*

Analysis	***Sample size***		***No. of different mothers***		***No. of mothers appearing in both categories (Litter reared solitarily / communally)***
	Litter reared		Litter reared		
	solitarily	communally	solitarily	communally	
Overall	24	30	20[1]	21[1]	7
Female nesting partners when non-reproducing[2]	8	8	8	8	2
Female nesting partners when pregnant[2]	12	13	12	13	3
Individual associations[3]	13	31	12	16	3
Population density	24	30	20[1]	21[1]	7
Number of available options	24	30	20[1]	21[1]	7

*: For each parameter we compared cases where litters were reared solitarily despite the option to nurse communally ('litter reared solitarily') with those cases where litters were reared communally ('litter reared communally').
1: Mother assignments were not possible for 3 (reared solitarily), respectively 2 litters (reared communally).
2: Reduction in sample size due to the fact that only those pit-tagged females were included, who could clearly be defined as 'non-reproductive' or 'pregnant' and were not still nursing their previous litters.
3: Reduction in sample size resulting from the fact that only dyads with pit-tagged females were included, where females spent at least 5 common days in the barn (calculation of Fager's Index). Data resulted from 12 (reared solitarily), respectively 18 (reared communally) different litters.

Individual Associations

The associations between solitarily nursing mothers and her potential, but declined nursing partners, and the associations between communally nursing mothers and her nursing partners, differed significantly during the non-reproductive periods, with a higher spatial association present for the latter dyads (Mann-Whitney U-Test: $U = 107.5$, $P = 0.014$, Table 1 & Figure 3). In all cases except one, a communally nursing mother was also associated with her communal nursing partners during non-reproductive periods. Association indices varied between 0.09 and 0.71. Interestingly, close spatial associations during a female's non-reproductive period also occurred towards female conspecifics which were declined as communal nursing partners during reproduction (see Figure 3). Association indices of solitarily nursing females towards their potential nursing partners varied between zero and 0.68. Highest associations (indices of 0.26 to 0.67) towards females which were declined as nursing partners occurred between three pairs of founder females, which frequently shared their nesting sites but never nursed communally even though they had at least once the option to do so.

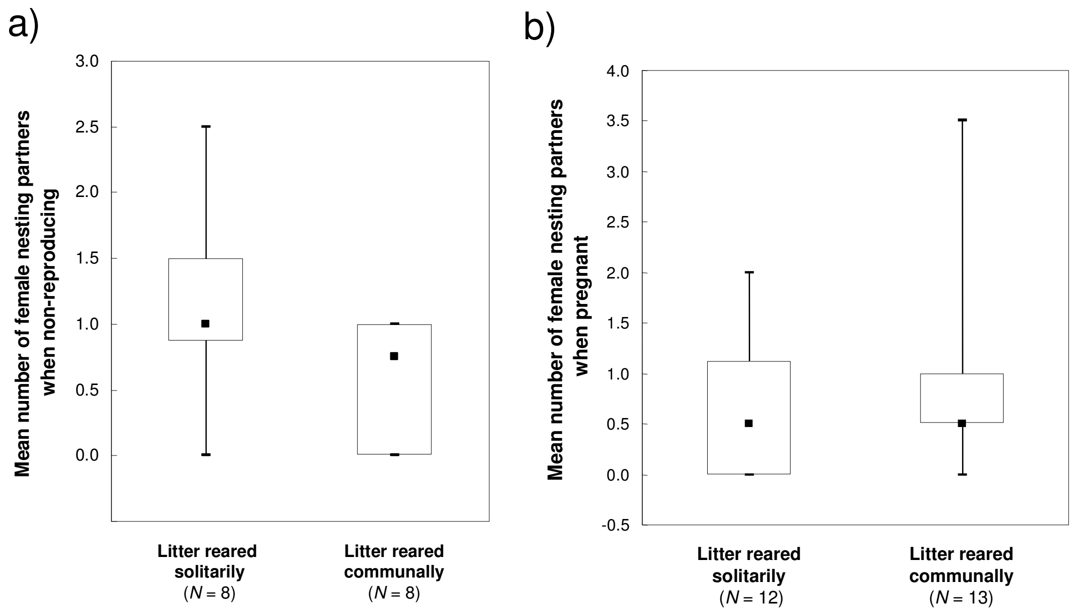

Figure 2 Mean number of female nesting partners a) during the non-reproductive period and b) during pregnancy for prospective mothers who reared their litters solitarily despite the option to nurse communally, and those who reared their litters communally. Median, box: interquartile range 25%-75%, whiskers: Min.-Max..

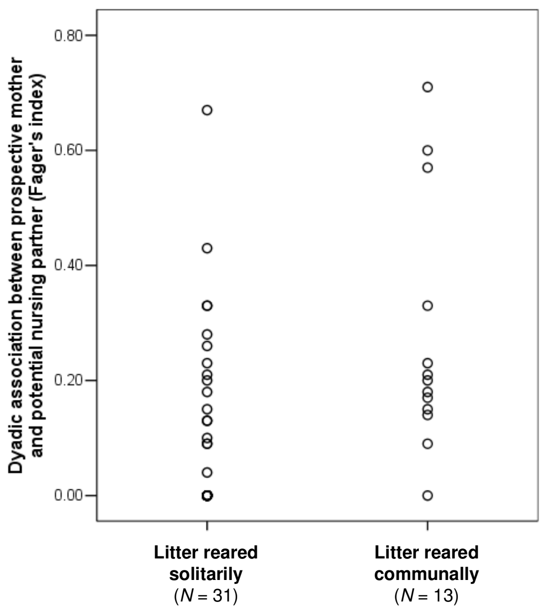

Figure 3 Individual associations of prospective mothers to their potential nursing partners during the mothers' non-reproductive period. Data are shown for cases where females reared their litters solitarily, despite the option to nurse communally, and for cases where females reared their litters communally. Dyadic associations are given for the mother towards all her potential nursing partners (litters reared solitarily) and towards all her taken partner(s) (litters reared communally), respectively.

Determinants of Communal Nursing Decisions

To investigate which determinants affect the decision to nurse communally, we compared population densities and the number of available nursing options between the 24 cases, where litters were reared communally and the 30 cases, where litters were reared solitarily, despite communal nursing options. Interestingly, population density did not significantly affect the probability that an option to nurse communally was used ($F_{1,52}$ = 1.55, P = 0.22, Table 1 & Figure 4a). Decisions to nurse communally with other females were already made at very low densities with just a few animals living inside the barn area. Just as well, litters were reared solitarily at maximum density of 0.94 individuals per square meter, despite the option to nurse communally. In contrast, communal nursing decisions were significantly affected by the number of available communal nursing options ($F_{1,51}$ = 7.94, P = 0.007, Table 1 & Figure 4b). A greater range of available options led to a higher probability that at least one of those options was actually used. Nevertheless, litters eventually even ended up in solitary nests when their mothers had up to four options to nurse communally. There was no significant correlation between the population density and the number of available communal nursing options for those cases included in the model (Spearman's rho: 0.117, P = 0.40, N = 54).

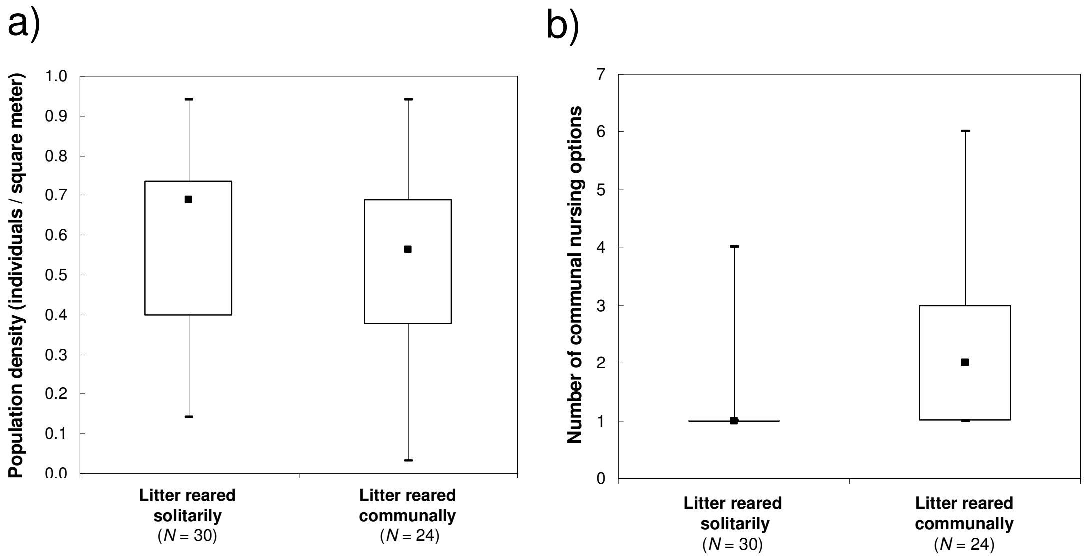

Figure 4 Communal nursing decisions (litter reared solitarily / communally) depending on a) population density and b) number of communal nursing options. Median, box: interquartile range 25%-75%, whiskers: Min.-Max..

DISCUSSION

Out data indicated that females can be choosy under natural conditions. Communal nursing decisions were influenced by the availability of partner options rather than by constraints of sociality. Female house mice did not use each option to nurse communally and communal use of nesting sites did not inevitably lead to communal nursing activities.

Number of Communal Nursing Partners

Overall, 69 of all 111 litters counted during the study period were reared solitarily, whereas 42 litters were reared in a communal nest. Two thirds (28 litters) of all litters which were nursed communally ended up in a nest with only one additional litter, meaning that in most of the cases, only two females nursed communally. One third (14 litters) contributed to communal nests consisting of three to four litters. Despite some exceptions, this finding is generally in accordance with expectations resulting from laboratory experiments where cooperative relationships were predicted to develop between only two reproducing females (König 1994a). Females in pairs had the highest reproductive success, whereas the females' reproductive success in groups of three was significantly lower than that of monogamous females, which raised litters on their own (König 1994a). Also theoretical models, predicting that cooperation based on mutualism or reciprocity is dependent on stable pairs (Boyd & Richerson 1988), point in the same direction.

Communal Nursing Options, Nest Mates & Individual Associations

64 out of the 111 litters had at least one option to nurse communally and only those cases can be sensibly used for the further analysis to understand and interpret communal nursing decisions. Interestingly, in only 24 of those 64 cases the option to communally nurse was taken and in 30 cases litters were nursed solitarily despite the option to nurse communally. Seven mothers displayed both nursing decisions and established communal and solitary nests with successive litters.

The fact, that mothers may change their use of options over time suggests that individual females do not generally follow a single nursing strategy that is either nursing communally or solitarily. Communal nursing decisions rather appeared to be made independently for each litter born, depending on the present circumstances.

In our study population, the option to nurse communally was not always used. Solitary nests occurred, despite the option to nurse communally. Often, the mothers had not only one, but even up to four options to establish a communal nest. We could therefore not support previous findings by Manning et al. (1992), suggesting that communal nursing is an obligatory behaviour. In contrast to our study, however, the study of Manning and colleagues was conducted in semi-natural enclosures where dispersal was not possible, a situation which could have affected the females' nursing decisions. Females, which would have rather left the group and reared their litters solitarily or with another partner, might have been constrained to nurse communally.

So far, we have shown that communal nursing is not obligatory. However, this does not necessarily indicate that females can be choosy in their communal nursing decisions. An alternative explanation for not-using communal nursing options is that females ending up in a single nest were generally less social and tended to segregate from other group members already before reproduction. This was not the case in our study population. Mothers who differed in their communal nursing decisions did not differ in their sociability concerning the number of female group members prior to reproduction. Even during pregnancy, the number of female nest-mates did not differ between those prospective mothers which nursed solitarily and those which nursed communally soon after, which suggests that omitting nursing options during reproduction might indeed be a female decision.

This suggestion could finally be confirmed by our results on individual spatial associations between prospective mothers and their potential nursing partners. Females, which were spatially associated outside the reproductive period, did not necessarily nurse communally when it came to reproduction. In opposition to previous predictions (Manning et al. 1995; Hayes 2000), this indicates that communal nursing is no unavoidable by-product of sharing nesting sites in female wild house mice. Nevertheless, close spatial proximity during non-reproduction might still be inevitably linked to communal nursing activities in other species, such as for example in lions (Pusey & Packer 1994) where non-offspring nursing appears as a by-product of communal cub defense. In addition, our results do not only argue against communal nursing as a side effect of nest sharing but also indicates that nest-sharing and communal nursing are two separate decisions which may even be subject to different selective regimes (also see Hayes 2000). This becomes especially obvious in founder females, which frequently shared nesting sites but never nursed communally even though they had the option to do so. Indeed, requirements for nesting and nursing partners might differ and appropriate nesting partners might not necessarily serve as appropriate social partners for communal nursing activities. It is thus not unlikely that individuals may display different partner preferences in different social contexts, as stated by Dugatkin and Sih (1995). This notion is already supported by results of a recent lab experiment, where female house mice changed their preferences for social partners when mating opportunities and thus the necessity to choose communal nursing partners were perceived to be proximate (Weidt et al. in press).

Nevertheless, even though close spatial proximity did not necessarily lead to communal nursing, all communally nursing females except one shared common nesting sites, at least for some days, during the non-reproductive period. Individual associations between females which shared a communal nest were significantly higher than individual associations between prospective mothers and their potential, but declined options. This is not surprising, as close spatial associations are assumed to express social partner preferences, a relation suggested in several previous studies on house mice (Wilkinson & Baker 1988; Manning et al. 1995; Dobson et al. 2000; Hayes 2000; Rusu et al. 2004; Weidt et al. in press). In short, even though communal nursing is generally linked to spatial proximity, spatial associations during non-reproduction do not neccessarily lead to communal nursing.

In three cases, one and the same potential nursing partner was once taken but another time omitted by the same prospective mother. This interesting side observation showed that there was no general decision for or against specific individuals as communal nursing partners, and that communal nursing decisions may differ over time, probably depending on an individual's and the potential partner's status and condition, as well as on social and ecological conditions.

Determinants of Communal Nursing Decisions

Communal nursing is not obligatory and does not occur as a by-product of close spatial proximity. Nevertheless, the probability of using a communal nursing option may still be influenced by parameters linked to group life. Many authors have suggested that social behaviour is affected by population density (e.g. Brown 1974; Emlen 1982). According to the habitat saturation hypothesis (Getz et al. 1992) individuals cooperate when resources, such as food or appropriate nesting sites become limiting at high population densities. Furthermore, an increase in population density might also enforce competition and thus for example increase the risk of infanticide, making it additionally profitable to share and defend nests with others. Interestingly, we found no effect of density on the probability to use a communal nursing option in our study. On the one hand, options to nurse communally were already used at very low densities, but on the other hand, options were as well declined at highest densities in our study population. Even though population densities reached a maximum value of nearly one individual per square meter, scarcity of resources did not seem to be prevalent, at least food and water was always available ad libitum.

However, the probability to nurse communally increased with an increasing number of available nursing options. In female house mice, the choice of an appropriate social partner is crucial for the development of egalitarian reproductive relationship and social partner choice thus results in significant fitness consequences (Weidt et al. in press). A higher number of potential nursing partners increases the probability to meet an appropriate partner for communal nursing activities. This can well explain the effect of the number of potential nursing partners on communal nursing decisions, and offers, again, strong support for the idea that females are choosy in their partner choice decisions. The probability to nurse communally is predominantly influenced by the likelihood to meet an appropriate social partner. Nevertheless, females may still decide to nurse solitarily, even when having up to four options to establish a communal nest.

Indeed, females have a choice! Our results showed that communal nursing is not an obligatory behaviour emerging as an unavoidable by product of nest-sharing. The probability to use a communal nursing option though was significantly affected by the number of potential nursing partners, suggesting that the availability of appropriate cooperation partners is indeed crucial and strongly determines communal nursing decisions.

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CHAPTER 4

Minimizing competition & maximizing benefits?

A study on the coordination of reproductive cycles in female wild house mice

to be submitted

Minimizing competition & maximizing benefits?
A study on the coordination of reproductive cycles in female wild house mice

ANDREA WEIDT & BARBARA KÖNIG

Institute of Zoology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Cooperation partners should minimize competition and maximize benefits from cooperative interactions. In house mice (*Mus domesticus*), females may reach this aim when reproductive cycles of social partners are not fully synchronized. On the one hand can females reduce mate-competition if same-sex group members are not receptive at the same time. On the other hand can communally nursing females avoid peak loads during lactation and thus increase their energetic benefits when litters are several days apart in age. We therefore predicted that potential cooperation partners coordinate reproductive cycles in a way that they are not fully synchronized. We analyzed vaginal smears in groups of virgin house mouse sisters and characterized cycle phase and cycle length in the absence of males in the first part of the study. In the second part, we described the reproductive cycle of sister-pairs in the presence of males. We finally investigated if female sisters indeed coordinate their cycles in the presence of males in the third part. Cycle states varied greatly among and between groups and individual cycle lengths between and among individuals in the absence of males. Many females never exhibited an estrous state prior to male exposure. The introduction of males, however, clearly increased the frequency of estrous events and cycle alternations occurred. Nevertheless, the frequency of cycle alternations in sister pairs housed together did not differ from hypothetical cycle alternations of a control group, singly housed sisters. Overall, we did not find an indication that prospective cooperation partners coordinate their reproductive cycles to minimize competition and maximize energetic benefits. Most probably, the selective pressure on females for actively coordinating their reproductive cycles is very low. Rather than actively coordinating, females may actually choose appropriate cooperation partners. Furthermore, other cues than estrous synchrony, such as MHC-characteristics or cues signaling reproductive activity are most likely of higher importance in such social partner choice.

Keywords

Competition, cooperation, coordination, energetic benefits, estrous alternation, house mice, *Mus domesticus*, peak-load reduction, reproductive cycles

INTRODUCTION

Cooperation among group members is frequent in animal societies, such as communal foraging or hunting (e.g. Boesch 1994; Creel & Creel 1995), communal nest defense (e.g. Bosque & Molina 2002), or cooperative breeding among females. Cooperative breeding appears as joint nesting or sharing of brood rearing duties (e.g. Vehrencamp 1977; Willmer 1985; Bernasconi & Strassmann 1999; Öst et al. 2003), and may even extend to communally nursing young (reviewed in Packer et al. 1992; Hayes 2000).

Still, cooperation partners are also competitors for essential resources, such as food, nest-sites or mates. In general, group life is only selected if its positive effects on reproductive success outweigh the negative effects of proximity to competitors. We therefore expect that especially cooperative relationships are characterized by rather low competition, where cooperation partners maximize the benefits of such interactions.

We investigated these aspects concerning the reproductive coordination of potential cooperation partners in female wild house mice (*Mus domesticus*). House mice are a well-studied rodent, where females may cooperate by communally nursing their young (e.g. Sayler & Salmon 1969; Baker 1981; Wilkinson & Baker 1988; König 1989). There is already evidence that females choose such same-sex group members as cooperation partners with whom they do not strongly compete for reproduction (Weidt et al. in press). House mouse groups are characterised by one territorial male, few, if any, subordinate males and several breeding and non-breeding females (Reimer & Petras 1967; Selander 1970; Lidicker 1976; Mackintosh 1981; Anderson & Hill 1965).

Wild house mice have no distinct mating season and females display reproductive cycles throughout the year. Reproductive cycles are generally rather short (average 4-6 days, e.g. Allen 1922; Parkes 1928; Drickamer 1992) and receptivity usually prevails for only one night. According to mating observations, a male often guards a female the whole night and inseminates her just at the end of the perceptive period, shortly before dawn (Sven Krackow, personal communication). By this kind of mate guarding, a male might ensure that he is the only father of the progeny. On the female side, however, this male behaviour may result in high female-female competition for mating partners, especially so when the number of males are limited and females are receptive at the same time and thus risk to miss fertilization (Rusu & Krackow 2004). However, mate-competition is reduced, if female reproductive cycles are not synchronized and female group members can mate sequentially with the same male.

Under favorable conditions young mice are sexually mature at 6-8 weeks and females can rear a litter of 4-9 young every month. Lactation is energetically costly, and females reach peak loads when pups are 13-16 days old, shortly before weaning (König & Markl 1987).

However, there is evidence that communally nursing females can avoid those peak loads when litters are several days apart in age (Müller 2001; König 2006). Under those circumstances, when births of cooperating females are not synchronised, can females thus maximize energetic benefits during cooperation.

House mouse mating behaviour and the potential for peak load reduction suggest that females minimize competition and maximize energetic benefits if reproductive cycles and the birth of litters in a communal nest are not synchronized, but shifted by several days. In a number of mammalian species, e.g. in primates and rodents, it has been reported that females within a group coordinate their reproductive cycles. Often, female group members synchronize their reproduction (see Kappeler & van Schaik 2004 for a review on causes and consequences of reproductive synchrony), but female chimpanzees at Mahale for example avoid synchronizing their estrous periods, presumably due to the very reason mentioned above, to decrease female-female competition for mates (Matsumoto-Oda et al. 2007). In house mice, we also expected females to coordinate their cycles. We predicted cycle asynchrony between prospective cooperation partners, which should result in reduced competition and increased energetic benefits. We focussed on familiar sisters as they almost always establish cooperative relationships (König 1994, 2006). Reproductive coordination, if present, is thus expected to be most prominent among sisters.

As no information on the timing and the conditions of possible cycle coordination was available, we first investigated the reproductive cycles of sisters in groups of two to four individuals. We then proceeded with analyzing the cycle development of sister dyads in the presence of males in the second part of the experiment. We finally compared cycle synchrony of females in pairs of sisters housed together, with the hypothetical cycle synchrony of two sisters housed solitarily.

METHODS

Animals and Husbandry

Animals were direct descendants of wild-caught and randomly bred house mice, *Mus domesticus*, originating from five geographically separated wild populations in the vicinity of Zürich, Switzerland (all populations shared the same karyotype, 2n = 24).

Mice were housed in Macrolon-cages on standard animal bedding, with food (laboratory animal diet for mice and rats, no. 3804 & 3336, Provimi Kliba SA, Kaiseraugst, Switzerland), water and nest building material *ad libitum*. Pups were separated from their parents at the age of 23 days and kept in separate cages with same-sex litter mates. Animals were kept under standard laboratory conditions throughout the experiment (14:10h light:dark cycle, one hour twilight at the beginning and end of the light phase; 22±1 °C, 50-60% relative humidity).

Determination of the Females' Reproductive Cycles

First, we examined the females' external vaginal morphology. We distinguished between females where the vagina was sealed to the outside, further referred to as 'closed', and females that showed a vaginal opening, further referred to as 'open'. Whenever females had an open vagina, we used vaginal smears to further assess the phase of the ovarian cycle. Vaginal smears were collected with a sterile platinum-loop, processed with Hemalaun-Eosin staining (approx. 1.5 % Eosin, 48.5 % distilled water, 50 % Ethanol, 10 min), and analyzed by light microscopy (Leica, Germany). The ovarian cycle was classified into four phases: proestrous, estrous, metestrous and diestrous, according to Allen (e.g. 1922). Briefly, proestrous smears contain only nucleated and cornified cells, estrous smears consist of exclusively cornified cells, metestrous smears contain large numbers of leukocytes in clumps or dispersed in smeary mucous with nucleated epithelial cells and some cornified cells, whereas during diestrous, although leukocytes where the predominant cell-type, they were less abundant than during metestrous, and they were accompanied by few epithelial nucleated and cornified cells, pleomorphic cells, as well as large dark stained nuclei (Fig. 1).

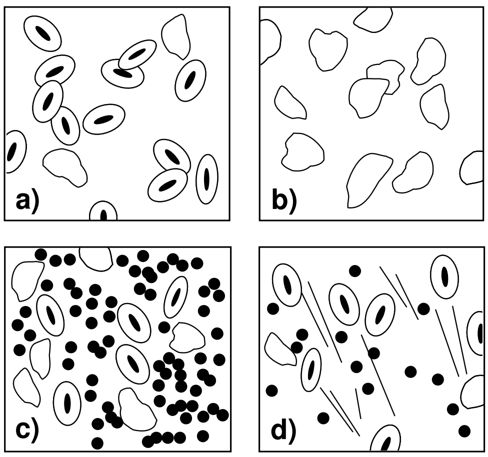

Fig. 1 Identification of the estrous cycle phases. Cellular content in vaginal smears of house mice in (a) proestrous, (b) estrous, (c) metestrous and (d) diestrous phases. See text for explanations. Simplified drawing according to photos in Meziane et al. (2007).

For females with an open vagina, we classified them as 'cycling' or 'non-cycling'. Individuals were defined as 'non-cycling' when the vagina was open, but either no estrous occurred throughout the sampling time or only one estrous so that no cycle could be determined. Only if at least two estrous events occurred, were females defined as 'cycling'. We defined individual cycle length as the mean number of days between two successive estrous phases (including the day of the second estrous) over all cycles during the sampling period. Whenever an individual was in estrous over several days, we used the interval between the last day of estrous until (including) the first day of the next one.

Throughout the study, individuals were generally only sampled every other day to reduce handling stress. We thus extrapolated the cycle states based on the existent information. Whenever a sampling gap was preceded by a diestrous and followed by a metestrous, we assumed that an estrous event has taken place. Contrary, if a sampling gap was preceded and followed by an estrous state, they were not counted as two separate events. In cycling females, several cycle states should occur in between two estrous events.

As a measure for cycle asynchrony, we analyzed the number of cycle alternations. We defined a cycle alternation as an event where two females rotationally showed an estrous state, e.g. female A was in estrous, female B in non-estrous, but the females' status was exactly alternated whenever the next estrous event occurred (female B was in estrous and female A in non-estrous). No cycle alternation occurred if both females simultaneously were in estrous or non-estrous, or if the status never alternated during the experiment (see Appendix I, group 6 for an example of cycle alternation).

Experimental Settings

Part 1: Reproductive cycles in groups of sisters in absence of males

Part one of the study was carried out during a period of 24 consecutive days. Females originating from 15 different litters were housed in Macrolon-III-cages (23.5 x 39 x 15 cm), with 1-3 of their same sex litter mates, without direct contact to males (males where kept in the same animal room, however not in neighboring cages). We collected data on the females' reproductive cycles on 40 virgin females (seven groups of 2 females each, six groups of 3 females each and two groups of 4 females each). At the start of the experiment, females weighed between 13.8 and 23.8 g and had an age of 53 to 154 days. Vaginal smears were collected between 15:00 and 19:00 h, and female body weight was taken once per week.

We analyzed the frequencies of female cycling categories ('closed' / 'open', 'cycling' / 'non-cycling'), and the distribution of individual cycle length. To investigate the influence of age, weight and number of cage-mates on a female's probability to cycle, we carried out a generalized linear mixed-effects model using a binomial error structure and the logit link function. The model was fitted using penalized quasi likelihood estimation (for details see Venables & Ripley 2002). We used the presence / absence of 'cycling' as the binary response variable, and age, weight and number of cage-mates as fixed factors. To account for potential effects due to same group origin, group identity was used as a random term. We based the analysis on type three sum of squares to investigate each term independently.

Part 2: Reproductive cycles in pairs of sisters in absence and presence of males

Following part one of the study, we investigated the development of reproductive cycles in six pairs of sisters from the first part of the experiment, now housed in two adjacent Macrolon-III-cages, linked by a tube. We investigated the females' ovarian phases over a period of 14 days in the absence of males, followed by 21 days where females' were exposed to the odor of a male, suggesting mating opportunity. We did this by attaching a third cage with an unfamiliar, unrelated male to the females' cages. A wire mesh in the tube connecting the male with the female cages allowed for olfactory and acoustic contact, but no mating. Again, cycle states of individual females were determined between 15:00 and 19:00 h, every other day.

To obtain information about the influence of male presence on the females' cycle development, we compared the mean number of estrous events per sampling day for each sister-pair in the absence and in the presence of males with a Wilcoxon Signed Ranks test. We further investigated the number of cycle alternations in the absence and in the presence of males.

Part 3: Coordination of female cycles in presence of males?

Part three of the study was carried out over a period of four weeks, with four groups of four sisters each. Sisters were litter mates and had an age of two to three months at the onset of the experiment. Two sisters each were housed together in two adjacent Macrolon-III-cages connected by a tube, while two were kept in separate Macrolon-III-cages and served as a control. All four sisters were simultaneously exposed to the same unfamiliar und unrelated male, where contact, but no mating between the sexes was possible due to a wire mesh partitioning the connecting tubes (see Fig. 2 for the experimental set-up).

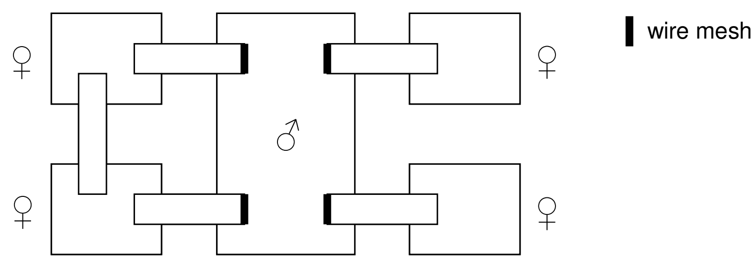

Fig. 2 Experimental set-up of part 3 of the experiment. Groups of four sisters, two housed together, the other two housed singly, were simultaneously exposed to a male for 21 days. Contact, but no actual mating was possible due to a wire mesh in the connecting tubes.

After seven days of female cohabitation, the cage with the male was connected for a period of 21 days. Throughout the time, each female's cycle state was determined between 15:00 and 19:00 h, every other day. All females had an open vagina at the start of the experiment.

To investigate if cohabitating females coordinate their cycles we compared the frequencies of initial cycle synchrony between sisters housed together and the control group, “hypothetically grouped” sisters housed in single cages with no physical and no immediate olfactory contact. We defined initial cycle synchrony as events where both females of a real or hypothetical dyad have their first estrous on the same day after male introduction. Furthermore, we compared the frequency of estrous alternations during male presence between sisters housed together and the control group.

Statistical Analyses

The statistical model was carried out using R for Windows, Version 2.2.0 (R Development Core Team 2005) and the packages ‘nlme’ (Pinheiro et al. 2004) and ‘MASS’ (Venables & Ripley 2002). Nonparametric statistics were conducted using SPSS 13.0. All tests were two-tailed and effects were regarded as significant at $P \leq 0.05$.

RESULTS

Part 1: Cycle States, Mean Individual Cycle Lengths and Cycling Probability

Eight of the 40 females were ‘closed’, they never showed an open vagina. Remarkably, 15 out of the 32 ‘open’ females also showed a sealed vagina on single or few successive days. Twenty females categorized as ‘open’ never showed an entire cycle with two estrous phases. Altogether 28 of 40 females were ‘non-cycling’ (Fig. 3). Nevertheless, 12 females were ‘cycling’, and cycle lengths varied largely between and among individuals, ranging from 4 to 10 days (see Fig. 3 for frequencies of mean individual cycle length). All cycling categories (‘open’ / ‘closed’ and among the open ones ‘cycling / ‘non-cycling’) could simultaneously appear in the same group of sisters, but sisters could also show the same cycling category.

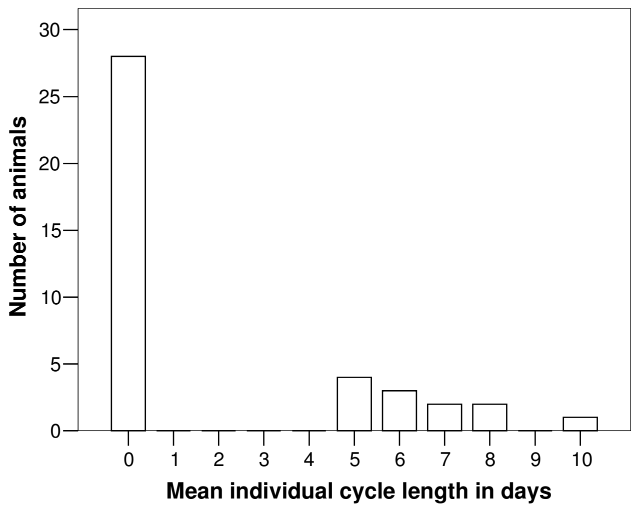

Fig. 3 Frequency distribution of mean individual cycle length (N = 40). For 28 females no cycle length could be determined. Eight females showed a closed vagina and 20 were open, but ‘non-cycling’. 12 females were cycling and showed mean cycle lengths between 5 and 10 days.

None of the three factors age, weight, or number of cage-mates significantly influenced a female's probability to cycle (age: $F_{1,11} = 1.66$, P = 0.22, weight: $F_{1,24} = 0.08$, P = 0.78, number of cage-mates: $F_{2,11} = 0.99$, P = 0.40).

Part 2: Frequency of Estrous Events and Cycle Alternations

The mean number of estrous events per sampling day increased significantly in the presence of males (Wilcoxon Signed Ranks test: Z = -2.2, P = 0.03, N = 6; Fig. 4). While an estrous phase only occurred with a median frequency per sampling day of 0.13 (Interquartile range (IQR): 0.38) prior to male introduction, estrous frequencies were much higher (0.88, IQR: 0.13) in the presence of males.

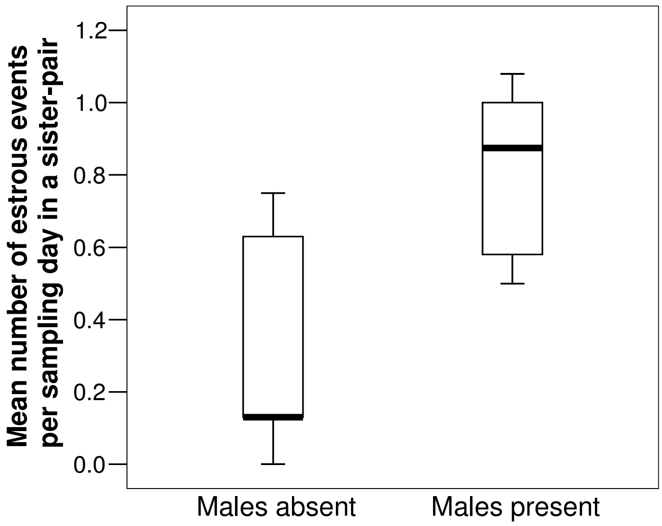

Fig. 4 Frequency of estrous phases in absence and presence of males. The mean number of estrous phases per sampling day in sister pairs (N = 6) were compared between the time period prior to and after male introduction. Median, box: interquartile range 25%-75%, whiskers: Min.-Max.

All 12 females were at least once in estrous until the end of the experimental period, and all except one was 'cycling'. Estrous alternation occurred in only one group prior to male introduction, whereas in five of six groups, females showed estrous alternation after a male was introduced. Alternation events ranged from one event to a maximum of nine alternations between the two females in the presence of a male (see Appendix I for detailed graphs on the females' cycles in the six sister-pairs).

Part 3: Initial Cycle Synchrony & Cycle Alternations

Initial cycle synchrony did only occur once, in one dyad of singly housed sisters, which came into first estrous on the same day after male introduction. The other three singly housed sister pairs as well as all four communally housed sister dyads showed no initial cycle synchrony. Cycle alternations between sisters housed together occurred in three of the four groups, with two groups showing one alternation each, and one group four alternations. Between the two singly housed sisters we found one hypothetical cycle alternation in three of the groups and two hypothetical alternations in the forth group if comparing their ovarian cycle states over time. Sisters housed in groups did thus not show an overall higher frequency of alternation events (see Appendix II for detailed graphs on the females' cycle in the sister groups).

Vaginal Plugs

Interestingly, we observed two females with altogether three vaginal plugs in the course of the experiment. The white, rather hard, cylindrical to pyramidal plugs sealed the vagina and dislodged after a while. Exemplarily investigation of one plug under the light microscope revealed that it consisted of huge amounts of cornified cells, as also apparent during estrous. One female exhibited a plug before the male was introduced, the other female showed two plugs after exposure to a male (nevertheless, mating was impossible).

DISCUSSION

Our experiments did not provide clear evidence that female wild house mice coordinate estrous cycles within groups to avoid synchronous reproduction.

No Reproductive Coordination among Sisters in the Absence of Males

The first part of the study intended to investigate if coordination of reproductive cycles takes place among sisters kept in groups without exposure to males. Overall, we could not detect any pattern of reproductive coordination at this stage of the females' development. We found large variation in reproductive cycle categories (20% of the females never had an 'open' vagina, and only about 30% of the females were 'cycling'), and in cycle length of females belonging to the same and to different litters. Furthermore, cycle states could neither be explained by weight or age, nor by the number of same-sex litter-mates.

The rather high number of irregular cycles and the great variation in cycle lengths might be considered as an artifact due to the experimental procedure of taking vaginal smears. This is, however, rather unlikely, as previous studies explicitly investigated this possibility and did not find an effect of the vaginal smear procedure on estrous cycle lengths (Drickamer 1992).

In addition, our results are in line with findings from the late 1950th, when Whitten (1959) already showed that the majority of females that were kept in groups in the absence of males did not exhibit regular estrous cycles. Furthermore, Whitten discovered that odors from conspecifics influence the timing of estrous cycles and of reproduction. While urinary chemosignals from other females retard female cycles, urinary chemosignals from males accelerate female cycles (Whitten 1958, 1959). Since then, several decades of research have produced a rather thorough understanding of the manner in which urinary chemosignals of male and female mice influence the sexual development and reproduction in females (see e.g. McKinney 1972; Vandenbergh et al. 1972; Drickamer 1982; Vandenbergh 1983; Drickamer 1992).

In the second part of the experiment we proceeded with introducing males (though separated by wire mesh) to pairs of sisters, expecting that this procedure induces estrous cycles, which would then offer the basis for studying potential coordination. Above all, females may only coordinate reproduction with potential cooperation partners when reproductive opportunities are perceived to be proximate, that is, when olfactory cues of a male are present.

The Importance of Male Presence for Investigating Female Reproductive Cycles

In line with early studies, showing that females in the presence of males overcome prolonged diestrous states and start cycling (Bronson & Whitten 1968; Marsden & Bronson 1964), all except one female was cycling and the mean number of estrous events per sampling day increased significantly in the presence of males. This indicates that reproductive cycles occurred with a higher frequency than prior to male introduction. Our results thus confirm the impact of males on female reproductive cycles and hence the importance of considering male presence for investigations on female cycle states. Furthermore, by inducing the females' reproductive cycles, which is accompanied by several changes in the individuals' hormonal system, male presence may also alter female preferences for social partners and thus impact female social partner choice (for further information on this topic see chapter 2).

In five out of six sister pairs did females show estrous alternations after the male was introduced, whereas estrous alternation only occurred in one pair prior to male introduction. This is surprising, as groups of females are reported to synchronize reproductive cycles in reaction to male exposure (Whitten-effect, Whitten 1958; Marsden & Bronson 1964). This finding could suggest that potential female cooperation partners alternate their reproductive cycles. However, this effect may also simply result from a generally increased frequency of cycling females. As females differ in their individual cycle lengths, reproductive cycles would soon appear as alternating after an initial synchronization due to male exposure. To demonstrate, that sisters actually coordinate reproductive cycles, we need to compare the reproductive cycles of sisters housed together with the reproductive cycles of a control group, consisting of two additional sisters that were housed singly and without direct olfactory contact to each other. Only if cohabitating sisters show a higher frequency of estrous alternations than the two sisters in the control group, can we assume that females indeed coordinate their reproductive cycles. We carried out such an experiment as a third step in our study.

No Reproductive Coordination among Sisters in the Presence of Males

If male presence induces estrous and communally housed females coordinate their cycles already from the very beginning on, we would expect a lower frequency of initial cycle synchrony in communally housed sisters. Even though one of four groups in singly housed sisters showed initial cycle synchrony, whereas this was not the case in any of the four groups in communally housed sisters (see Appendix II), this does not yet allow for assuming a possible coordination effect. Furthermore, comparing the frequency of cycle alternations between sisters housed together with hypothetical cycle alternations of sisters housed solitarily, we did not find an obvious difference. Even though we only had a low sample size for litters with four sisters, and statistical analysis was not possible, we would expect to see a pattern if coordination would take place. Overall, we did not find an indication that prospective cooperation partners coordinate their reproductive cycles to minimize competition and maximize energetic benefits.

Asynchrony in reproductive cycles was expected to benefit female house mice. Still, there may be no need to actively coordinate reproductive cycles between cooperation partners. In general, the probability for two females to show synchronized cycles is very low, especially if females cycle randomly. Furthermore, even if initial random cycling does not occur, that is when cycles are induced and become synchronized due to urinary chemosignals from males (Whitten 1958; Marsden & Bronson 1964), this does not necessarily need to result in continuous cycle synchrony. Cycle lengths differ among genetically very similar females, and even within individuals (as already shown by Allen 1922). Thus, reproductive cycles of two females, even when induced at the same time, are not expected to remain synchronized after male introduction, even if females do not actively de-synchronize. Overall, female synchronization of estrous cycles seems to be rather the exception than the rule.

Competition between females for mating partners due to synchronized receptive periods should therefore be scarce. In addition, females generally have access to more than one male under natural conditions (also shown by the occurrence of multiple paternities; Camani 2005; Dean et al. 2006) which further may reduce mate competition. This is especially true as mate preferences differ among females (Drickamer et al. 2000; 2003).

According to the ‘peak load reduction hypothesis’ (König 2006; Müller 2001) can cooperating females optimize their energy expenditure during lactation when communally nursing litters differ by several days in age. Given that females do not discriminate between own and non-offspring (König 1989) and allocate milk not according to own litter size but to the size of the communal nest (Neuhäusser-Wespy & König, unpublished observations), the energy budget of communally nursing females is assumed to remain on a rather constant and medium level, because both litters do not simultaneously reach the period of highest energy need. Still, active coordination of cycles in the sense of desynchronization is not a necessary prerequisite for litter age differences. If, as argued above, synchronized births are rather scarce, age differences of litters in a communal nest will be rather frequent.

Over all, the selective pressure on females to coordinate reproductive cycles may be low. Instead of actively coordinating reproductive cycles, females may rather choose appropriate cooperation partners. In fact, it was already shown that females are choosy and that social partner choice results in fitness benefits (Weidt et al. in press). If cycle asynchrony indeed matters, we would expect that females choose partners with shifted cycles and rather breed alone if all available potential partners show synchronous cycles (given that the costs of solitary breeding are not too high). However, it is also possible that other cues, such as for example MHC-characteristics (as suggested for mate choice, Drickamer et al. 2000; Gowaty et al. 2003; Penn 2002) or hormonal cues signaling reproductive ability are of higher importance than cycle asynchrony in female social partner choice.

'Mating Plugs' in Virgin Females

Vaginal plugs following mating are a common phenomenon (e.g. Baer et al. 2000; Dixson & Anderson 2002; Ramm et al. 2005). When ejaculation occurs and seminal vesicle fluid coagulates, such vaginal plugs are formed (Carballada & Esponda 1992). The presence of a vaginal plug, also referred to as 'mating plug', is used in studies with rats and mice to determine whether copulation occurred, being aware of the fact, that not all mating events necessarily result in a vaginal plug (e.g. Jemiolo & Novotny 1993; Chehab et al. 1997; Tilley et al. 1999; Keighren et al. 2003). Here we show that the presence of a vaginal plug may not necessarily allow for the conclusion that mating took place. Vaginal plugs can even occur in the absence of males, or when females are exposed to male cues, but in the absence of mating. The morphology and structure of such plugs was comparable to 'mating plugs', still, they were formed by female and not by male secretions. We cannot exclude that their development is a reaction to the experimental treatment of taking vaginal smears, but as far as we know, such a reaction was never reported up to date. Further investigations are necessary to understand under which conditions and for which purpose such 'non-mating plugs' are formed. Nevertheless, this finding calls for caution when using vaginal plugs as a clear indication for the occurrence of mating events.

ACKNOWLEDGMENTS

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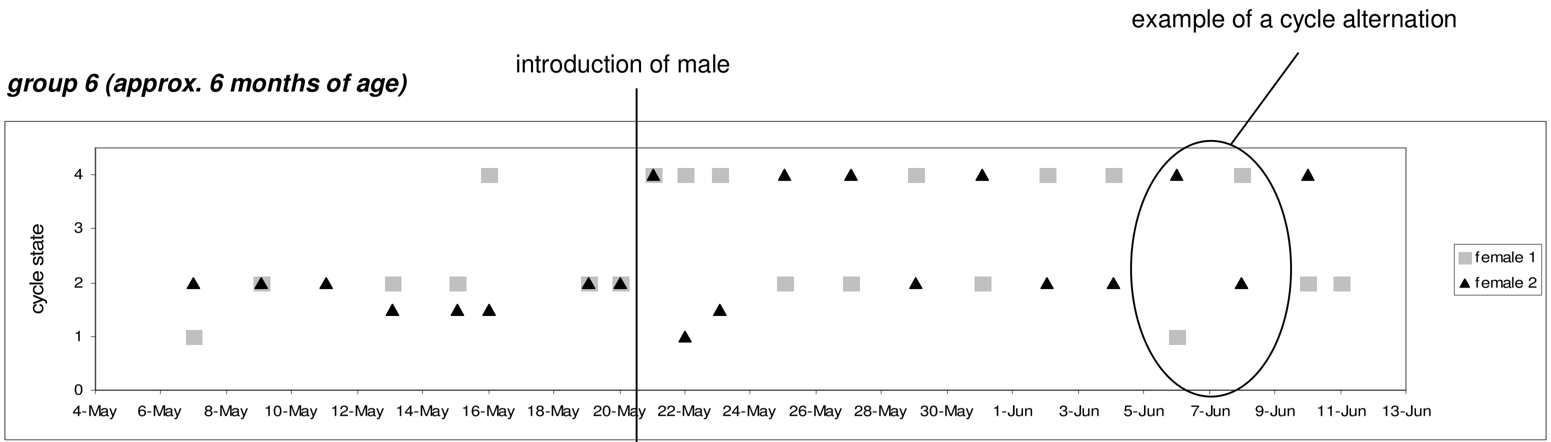
Appendix I

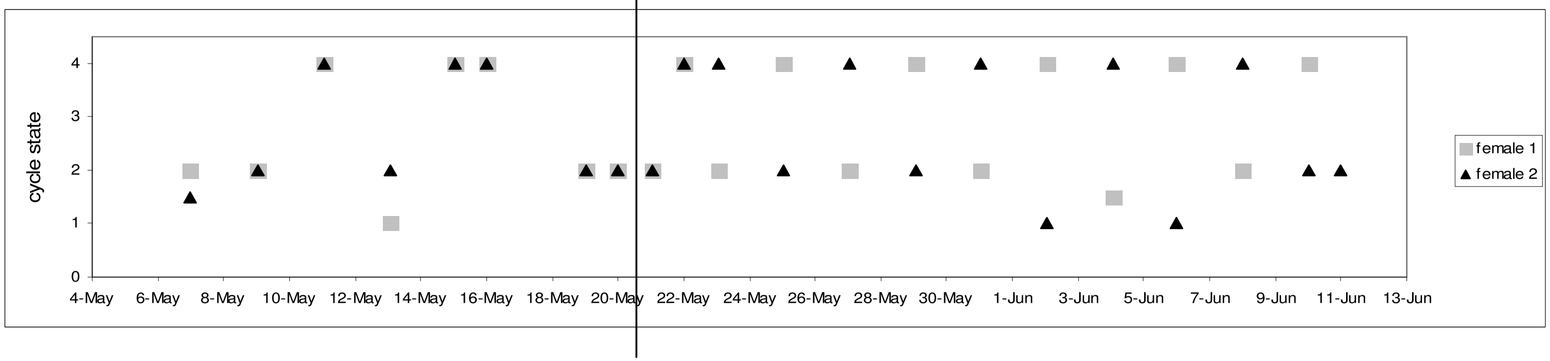
Cycle states of sister-pairs in part 2 of the experiment

cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous

group 6 (approx. 6 months of age)



group 8 (approx. 6 months of age)



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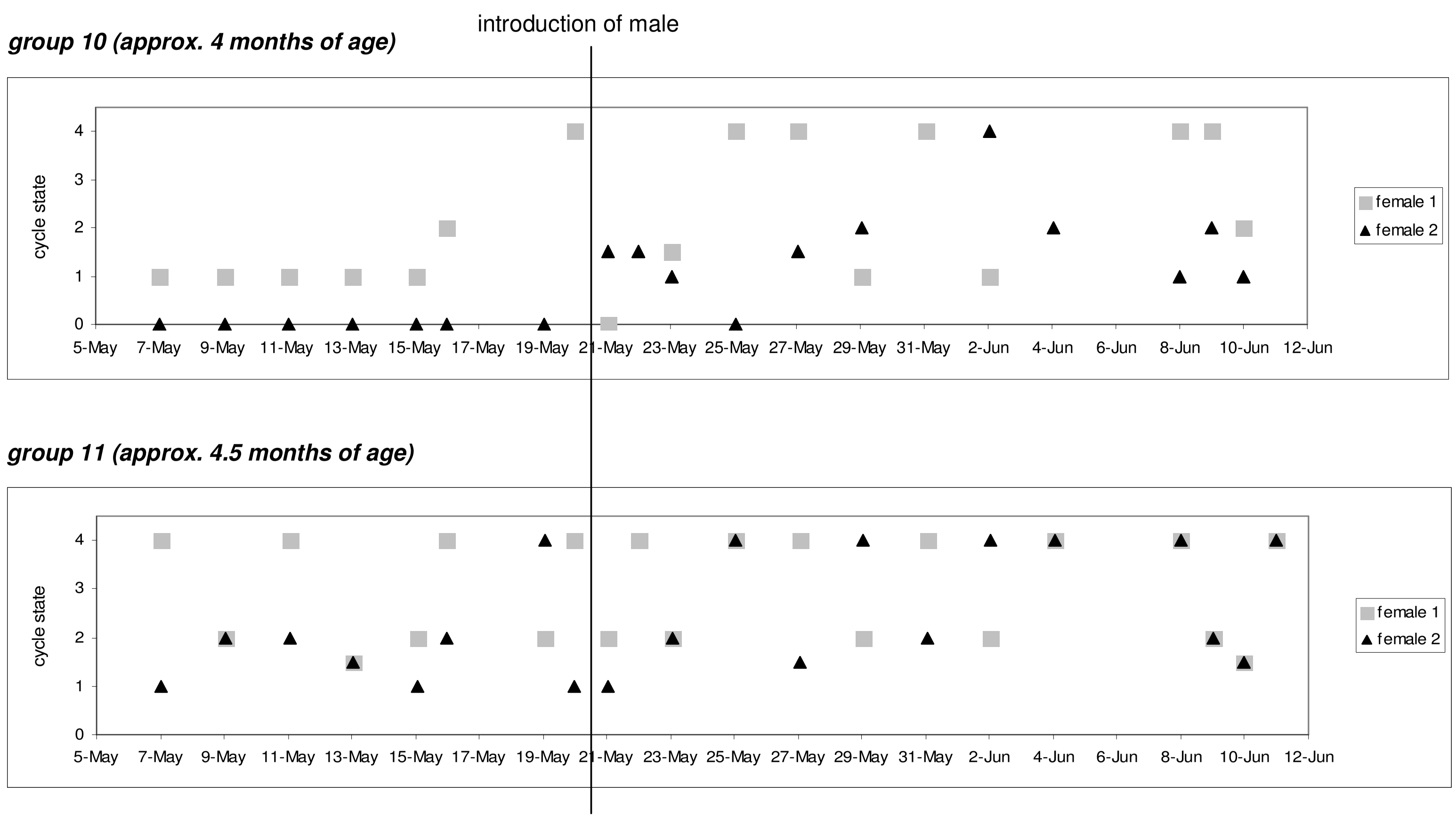
Cycle states of sister-pairs in part 2 of the experiment

cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous



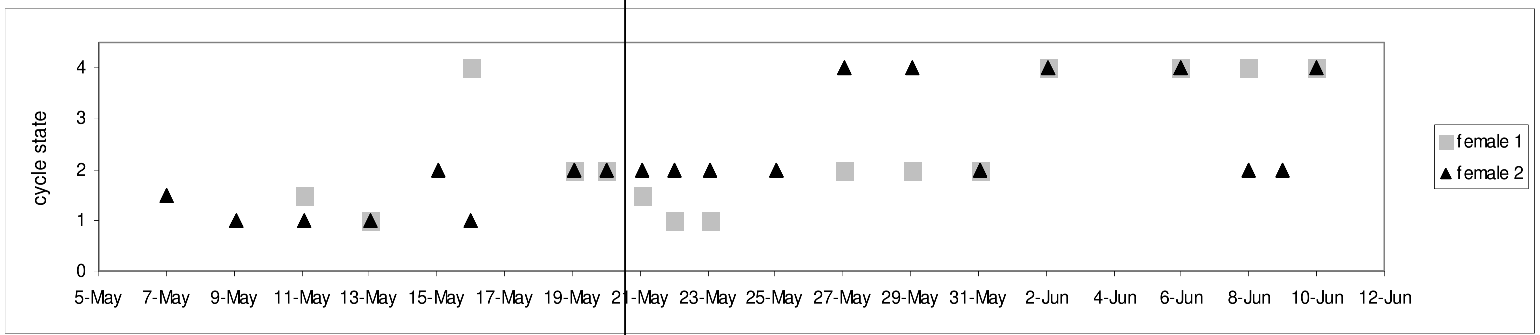
Appendix I

Cycle states of sister-pairs in part 2 of the experiment

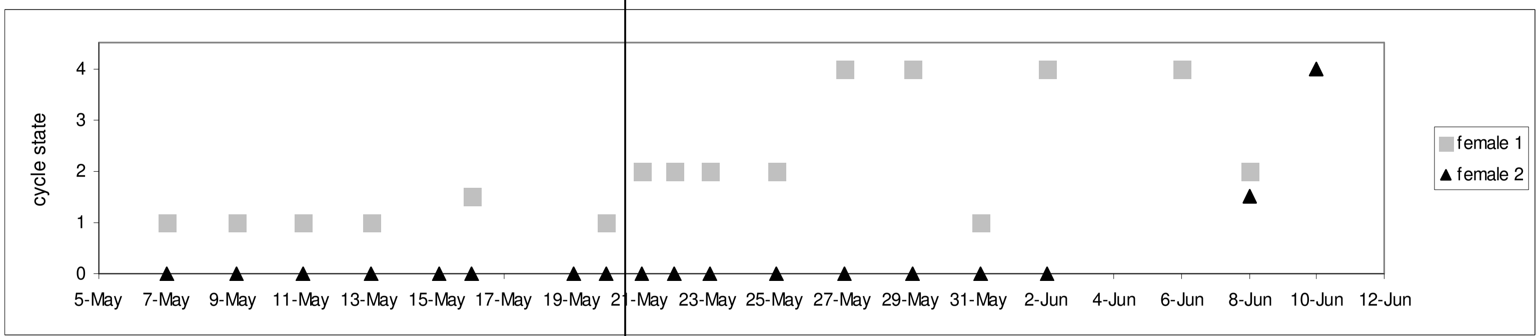
cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous

introduction of male

group 12 (approx. 4 months of age)



group 15 (approx. 4 months of age)



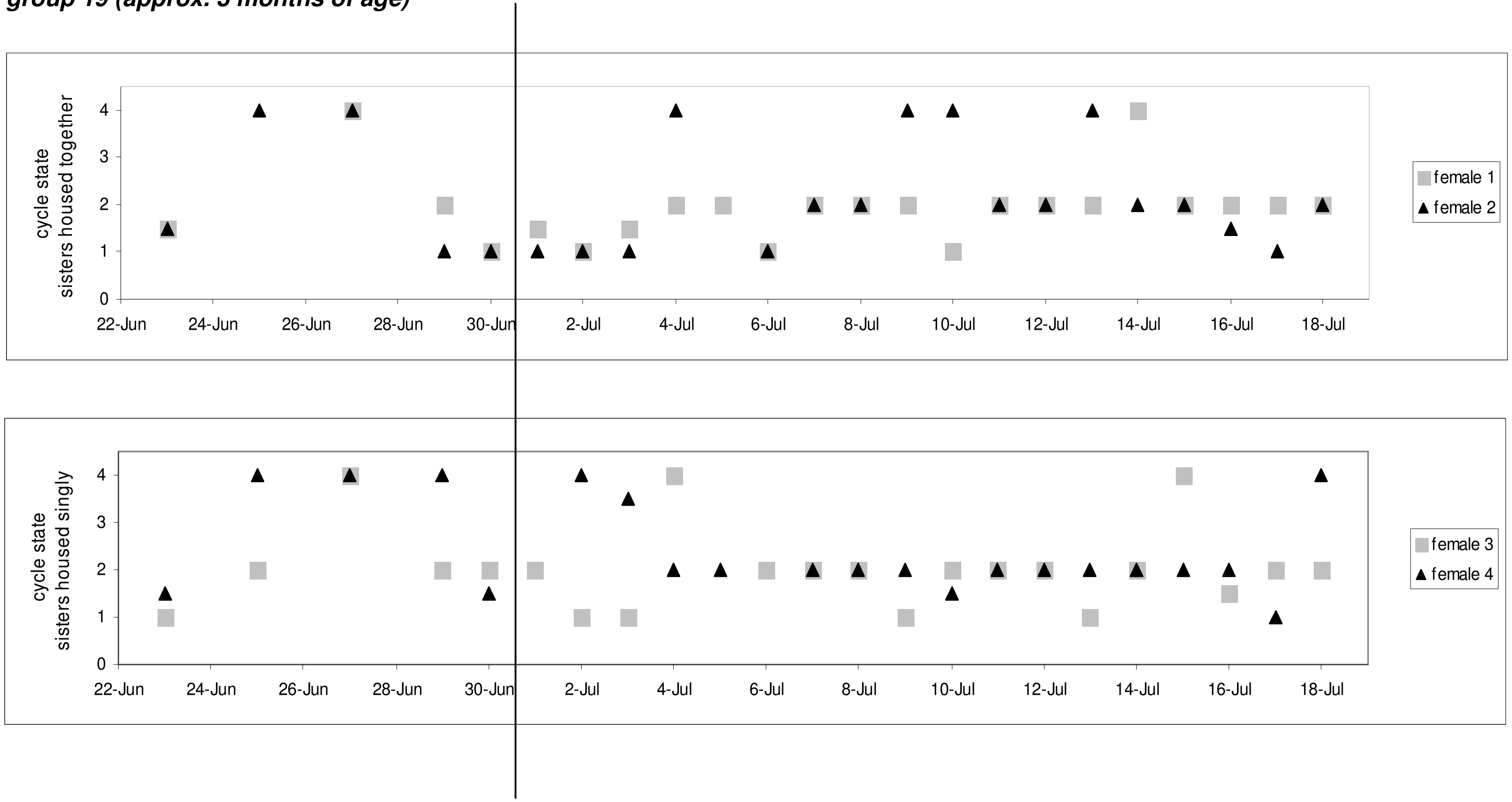
Appendix II

Cycle states of female groups in part 3 of the experiment

cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous

group 19 (approx. 3 months of age)

introduction of male

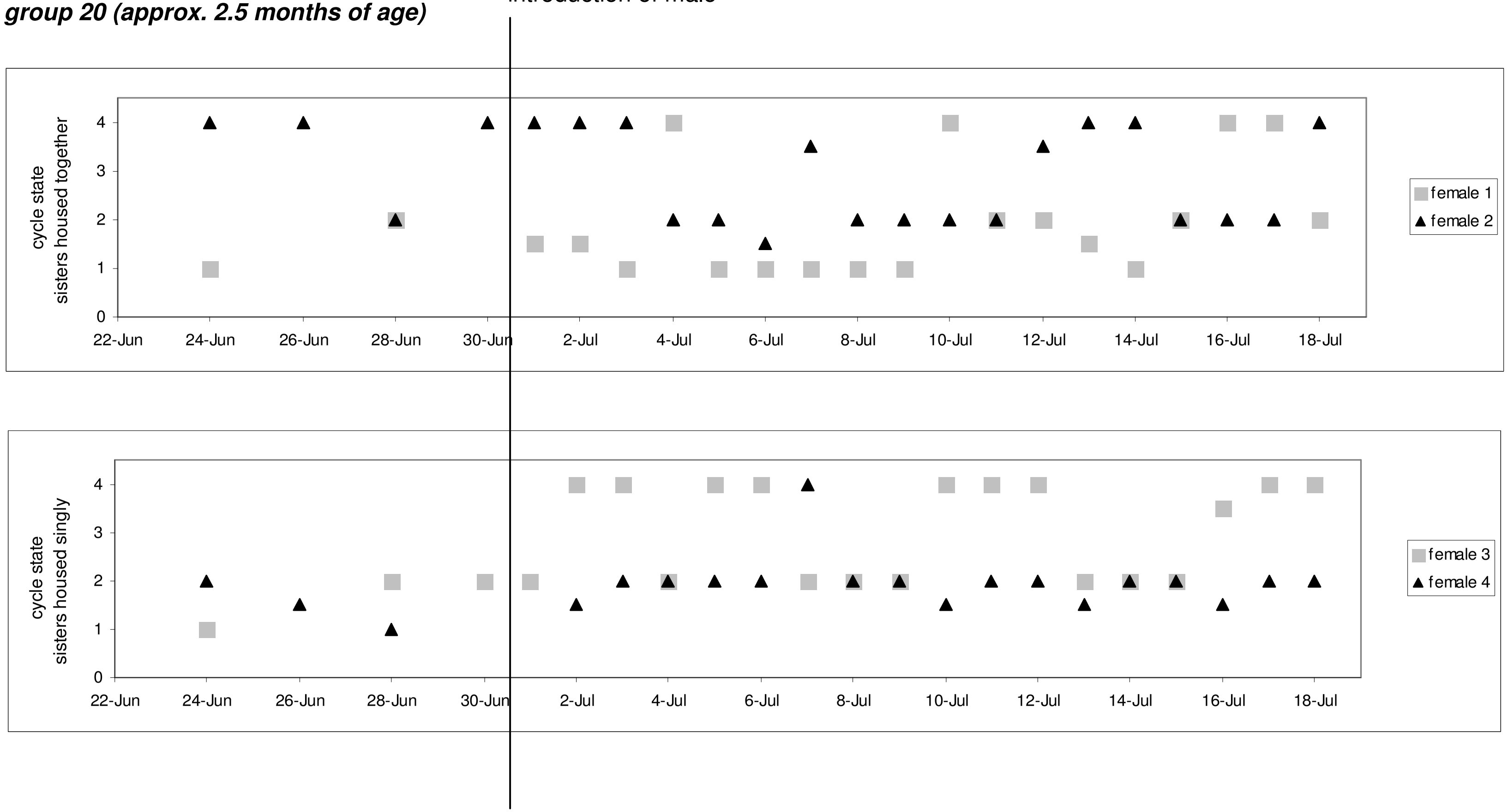


Appendix II

Cycle states of female groups in part 3 of the experiment

cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous

group 20 (approx. 2.5 months of age)

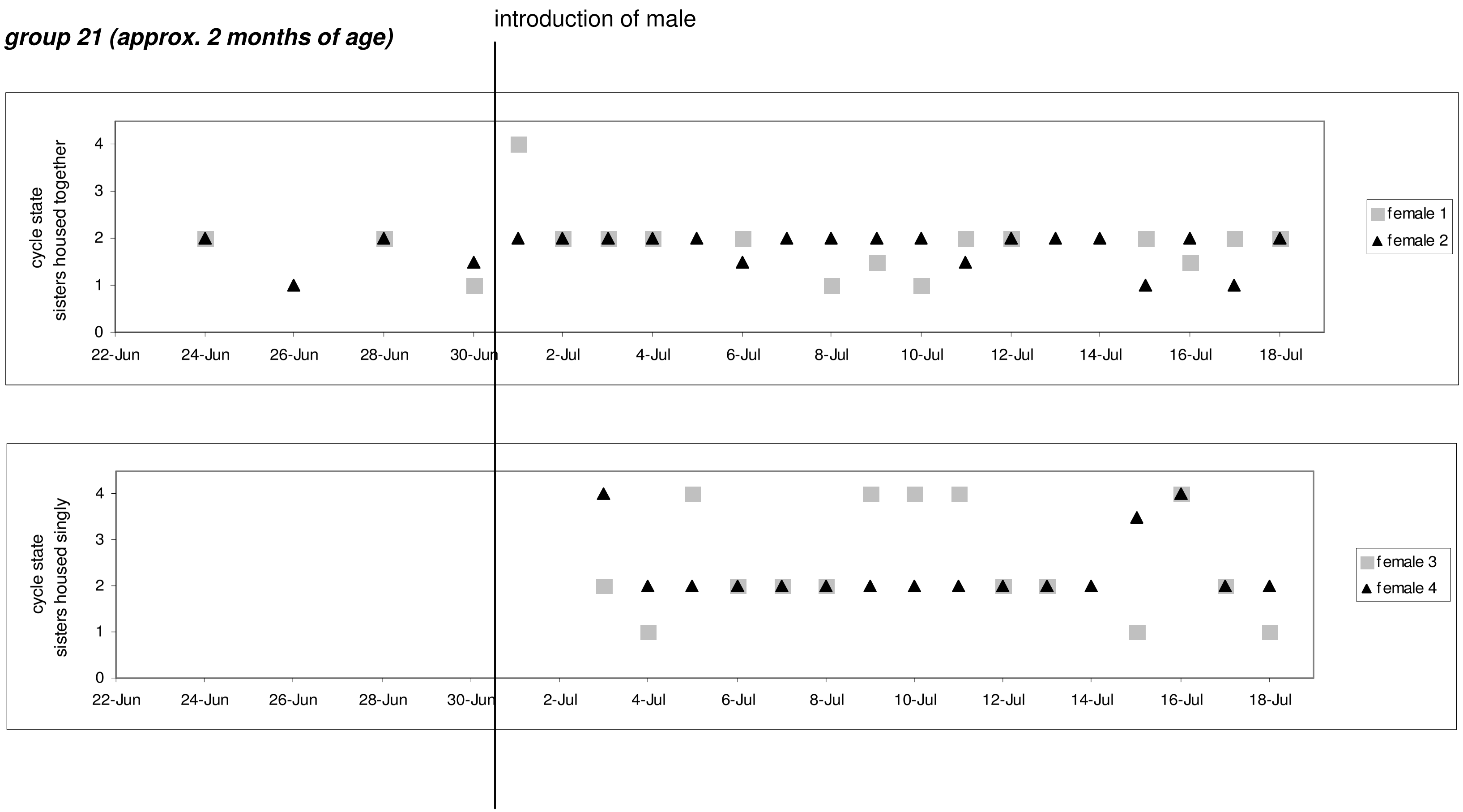


Appendix II

Cycle states of female groups in part 3 of the experiment

cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous

group 21 (approx. 2 months of age)

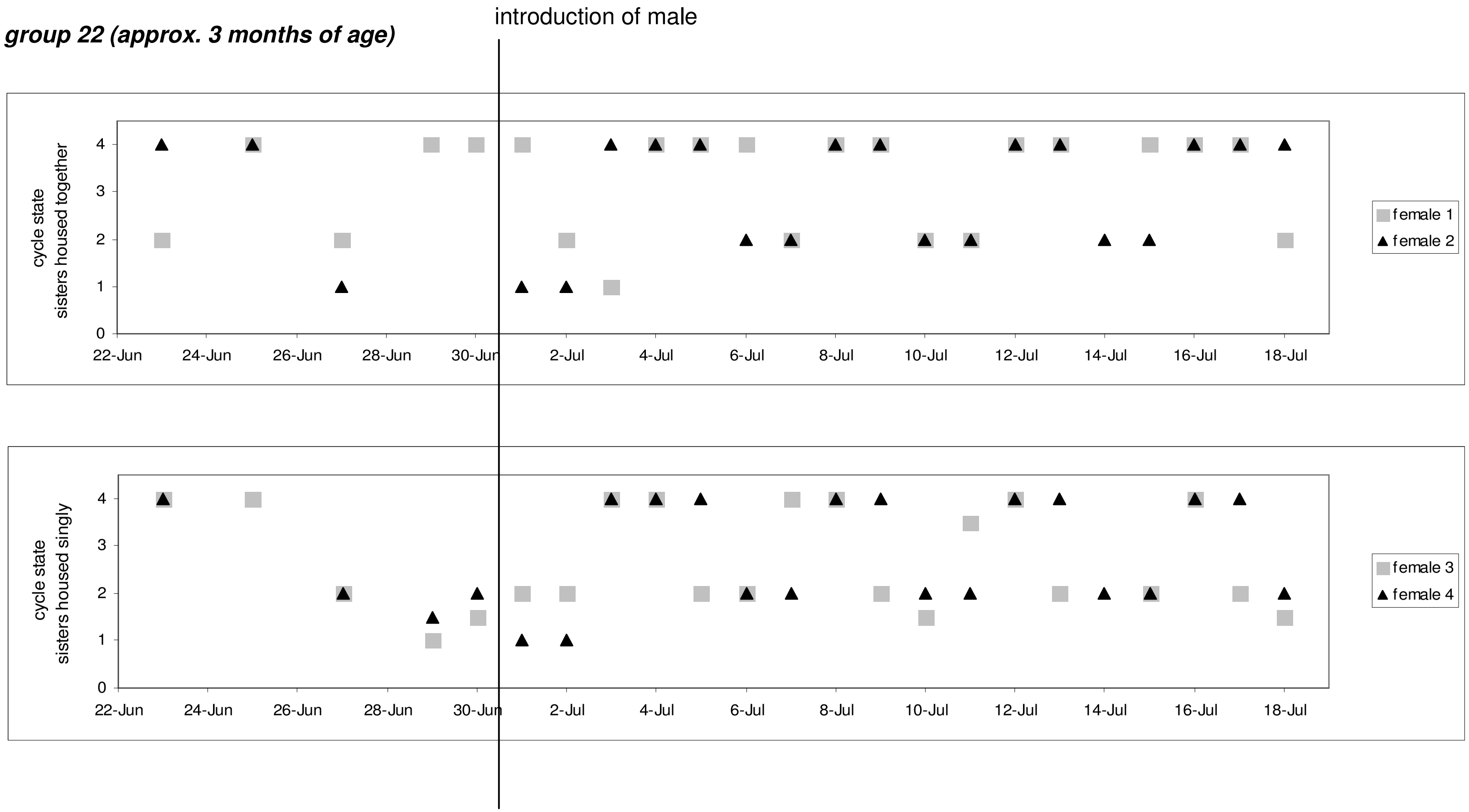


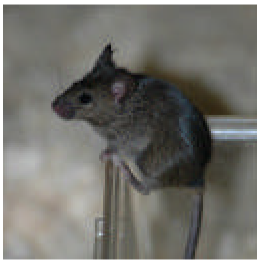
Appendix II

Cycle states of female groups in part 3 of the experiment

cycle states: 0 = vagina closed, 1 = diestrous, 2 = metestrous, 3 = proestrous, 4 = estrous

group 22 (approx. 3 months of age)





CHAPTER 5

A question of odor?

Major Urinary Proteins (MUPs) as possible cues in social partner choice among female wild house mice

A question of odor?
Major Urinary Proteins (MUPs) as possible cues in social partner choice among female wild house mice

ANDREA WEIDT, SAARA WELTI, BARBARA KÖNIG

Institute of Zoology, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Major Urinary Proteins (MUPs), a class of highly heterogeneous proteins in the mouse urine, carry species-, sex-, and individual-specific information. Preliminary data suggests that besides mediating individual recognition, MUPs may also play a role in social partner choice among female wild house mice. We investigated this topic in more detail, predicting that MUP-profile similarity is associated with female-female preference formation. We compared dyadic association indices with proportions of MUP-profile sharing (defined by isoelectric focusing) in groups of unfamiliar and unrelated female house mice in semi-natural enclosures. We found no indication that MUP-profile sharing is used as a cue in female social partner choice. However, due to methodological problems these results should be handled with caution. Future experiments on this topic are strongly advised 1) to be carried out in the presence of males, and 2) to use electrophoresis methods with a high resolution of MUP-bands, such as the Immobiline DryPlate system. The potential for MUP profiles as possible cues in female social partner choice and the necessity for further studies on their functional significance are briefly discussed.

Keywords

Associations, house mice, individuality signals, isoelectric focusing, Major Urinary Proteins, *Mus domesticus*, olfaction, partner preference, profile sharing, social partner choice

INTRODUCTION

Among mammals, odors are a primary mediator of individuality signals (Brown & Mcdonald 1985). In rodents a significant part of such olfactory individuality signals seems to be constituted by so called Major Urinary Proteins (MUPs), a class of highly heterogeneous proteins that bind and release small volatile pheromones when urine marks are deposited (Bacchini et al. 1992; Robertson et al. 1993; Beynon et al. 1999). In mice, over 99 % of the proteins excreted via urine are MUPs (Payne et al. 2001; Beynon & Hurst 2003). Besides playing an important role for individual recognition (Hurst et al. 2001), it is very likely that mice also use such olfactory cues in other circumstances where specific individual information could be crucial, for example when assessing and choosing potential social partners for cooperative activities.

Wild house mice, *Mus domesticus*, live in groups that are typically characterised by one territorial male, few, if any, subordinate males and several breeding and non-breeding females (Reimer & Petras 1967; Selander 1970; Lidicker 1976; Mackintosh 1981; Anderson & Hill 1965). Female house mice may cooperate by communally nursing their young (e.g. Sayler & Salmon 1969; Baker 1981; Wilkinson & Baker 1988; König 1989). Thereby females display non-random preferences for cooperation partners and such social partner choice yields significant fitness benefits (Weidt et al. in press). Social partner preferences are indicated by close spatial associations prior to reproduction (Dobson et al. 2000; Hayes 2000; Manning et al. 1995; Rusu et al. 2004; Wilkinson & Baker 1988; Weidt et al. in press). According to preliminary data from a pilot study on unfamiliar and unrelated female house mice, those spatial associations appeared to be facilitated by MUP-profile sharing (Rusu & Krackow, unpublished data; Rusu 2004, chapter 1 & 4). This suggests that MUPs, specifically the sharing of MUP profiles, may indeed play an important role during female-female spatial preference formation (Rusu 2004, chapter 4) and thus in female social partner choice.

In this study, we investigated this topic in more detail by comparing dyadic association indices with the proportion of shared MUP-profiles (defined by isoelectric focusing (IEF)) in groups of unfamiliar, unrelated female house mice interacting freely in semi-natural enclosures. We hypothesised, that MUP-profile sharing constitutes an important cue in female social partner choice in wild house mice. We specifically predicted that close associations are related to a high proportion of MUP-band sharing.

We focused on previously unfamiliar and genetically unrelated females to investigate a possible effect of MUP-profile similarity independent of kin-effects, which are known to influence the structuring of house mouse populations (e.g. Rusu & Krackow 2004). Under natural conditions, unfamiliar non-sisters represent a social category a maturing female may encounter when emigrating from their natal territory and either entering another group or forming a new group with previously unfamiliar und unrelated females (e.g. Baker 1981; Gerlach 1990, 1996).

METHODS

Animals and Husbandry

Animals were direct descendants of wild-caught and randomly bred house mice, *Mus domesticus*, originating from three geographically separated wild populations in the vicinity of Zürich, Switzerland (all populations shared the same karyotype, 2n = 24). Mice were kept under standard laboratory conditions (14:10h light:dark cycle, one hour twilight phase at beginning and end of the light phase; 22±1°C, 50-60% relative humidity).

Prior to the start of the experiment animals were housed with same-sex litter-mates in Macrolon-III-cages (23.5 x 39 x 15 cm) on standard animal bedding, with food (laboratory animal diet for mice and rats, no. 3804 & 3336, Provimi Kliba SA, Kaiseraugst, Switzerland), water and nest building material *ad libitum*. The experiment was carried out in indoor enclosures, which were 7 m^2 in size and surrounded by 80 cm high aluminium walls. Each enclosure was filled with standard animal bedding, equipped with six nest-boxes (15 cm diameter, 15 cm height), several PVC barriers for structuring, hay and paper towels as nest building material and three feeding and drinking sites.

Experimental Procedure

Between November 2004 and March 2005 we conducted 9 trails with six adult virgin females each (2-3 months of age). Within a trial, females were unfamiliar and genetically unrelated to each other and did not differ more than one month in age and 2 g in weight at the start of the experiment. All females were equipped with subcutaneously injected transponders (ID 100, TROVAN electronic identification systems) for individual identification. All six females of one trial were simultaneously introduced into the enclosure and kept in all-female groups for a period of 18 days. We collected data on the females' nest-box use and sampled urine from each individual female. In addition, all animals were carefully checked for scars or wounds once a week.

Dyadic Associations

For a period of 15 consecutive days, starting at day 4 after introduction, we registered the females' locations in the nest-boxes with a portable transponder reader once a day at midday (LID 500 Hand-Held Reader, TROVAN electronic identification systems). Dyadic associations between females of the same trial were determined according to the symmetrical index of Fager (Iij-index, modified by Kerth & König 1999). This association index, used as a measure of social partner preferences (see also Weidt et al. in press), may range from zero to one, where an association index of zero indicates that two individuals were never found in the same nest-box, an index of one that two females always shared the same nest-box.

Urine Collection

We collected urine from each individual female once between day four and day 18. Urine collection usually took place during weekly checks of body condition, were all females were removed from the enclosure and examined for scars and wounds. Individual females were placed in a blank PVC-box (56 cm x 37 cm, 40 cm high) where the animals usually urinated within a few minutes. Urine was collected with sterile one-way plastic pipettes and stored in glass tubes at -20℃ until further analyses of proteins were performed. The PVC-box was thoroughly cleaned between sampling events and new pipettes were used every time. Whenever animals did not urinate within a few minutes, they were placed back into the enclosure and the procedure was repeated on the subsequent days, until a decent sample could be collected.

Biochemical Analysis of MUPs

MUP profiles were determined by isoelectric focusing (IEF) gel electrophoresis. Prior to carrying out electrophoresis, we determined the ratio of protein/creatinine in each urine sample using a Coomassie Plus Assay (Bradford reagent and Albumin Standard, Pierce) and a Creatinine Assay (Picric acid solution, 1.3% saturated, Sigma, and Creatinine standard solution, 0.03 mg/ml, Sigma). Creatinine is excreted via urine at a constant rate and can be used as a metabolic standard. As the major part of urine proteins consists of MUPs (Payne et al. 2001; Beynon & Hurst 2003), total protein in relation to creatinine reflects MUP urine concentration. The proteine/creatinine ratio thus allows appropriate urine dilution for gel electrophoresis and therefore comparisons of MUP patterns.

Invitrogen IEF gel electrophoresis

For all 54 urine samples we carried out Invitrogen IEF gel electrophoresis in a XCell SureLock gel chamber (Invitrogen) on IEF Novex Gel (pH 3-7, Invitrogen), using IEF sample buffer (pH 3-7, Invitrogen), IEF marker (pH 3-10, Invitrogen) and IEF Anode and Cathode buffer (Invitrogen). All samples were diluted in ddH_2O prior to electrophoresis (10 µg protein in final volume of 5 µl). Gels were run at following conditions: step one at 100 V, 60 min, step two at 200 V, 60 min, and step three at 500 V, 30 min. Finally, IEF gels were fixed with 12% TCA fixing solution, stained with ProtoBlueSafe/EtOH mix and equilibrated in gel-dry solution. In general, each gel was loaded with one marker, one control sample (urine from a control individual not participating in the experiment) and six samples of experimental females.

Immobiline DryPlate gel electrophoresis

A few urine samples were additionally processed with Immobiline DryPlate gel electrophoresis, a more complex method in comparison to the Invitrogen IEF gel electrophoresis. Prior to electrophoresis, samples were diluted in rehydration buffer (10 µg protein in final volume of 5 µl). IEF gel electrophoresis was carried out in a Multiphor II

Electrophoresis System on Immobiline DryPlate gel (pH 4.2-4.9, Amersham Biosciences), using IEF electrode strips (Amersham Biosciences) and IEF marker (pH 3-10, Invitrogen). Gels were run at following conditions (if whole DryPlate gel is used): step one at 200 V, 5 mA, 15 W, 30 min, step two at 1000 V, 5 mA, 15 W, 30 min, and step three at 3500 V, 5 mA, 15 W, 4 h. Finally, IEF gels were fixed with 20 % TCA fixing solution, stained with coomassie stain and equilibrated in gel-dry solution. Again, each gel was generally loaded with one marker, one control sample and six samples of experimental females.

MUP-profile Sharing

Protein-bands were compared visually on IEF gels between all females of one group. We investigated the proportion of MUP-band sharing between two females by counting the number of bands present in both individuals in relation to the total number of different bands present in this dyad (see Fig. 1).

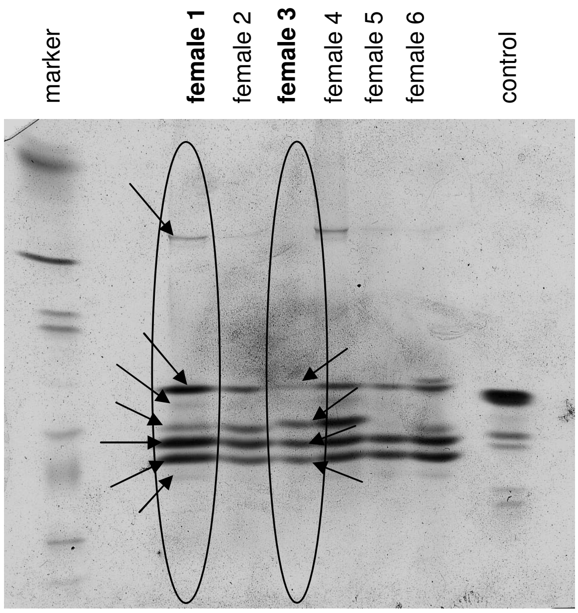


Fig. 1 Example of IEF gel after Invitrogen IEF gel electrophoresis. Protein-bands and the proportion of MUP-band sharing between two females are distinguished visually. For example, the proportion of MUP-band sharing between female 1 and 3 is 0.57 (female 1and 3 shared four out of seven bands).

To analyze whether spatial proximity, and thus preference, can be explained by similarities in MUP profiles, we carried out a linear mixed-effects model (Venables & Ripley 2002) fitted by residual maximum likelihood. We used the association index as the response variable, the proportion of MUP-band sharing based on Invitrogen IEF gel electrophoresis as the explanatory variable and incorporated group identity as a random term to account for possible effects due to same group origin.

Statistical Analyses

The statistical model was carried out using R for Windows, Version 2.2.0 (R Development Core Team 2005) and the packages 'nlme' (Pinheiro et al. 2004) and 'MASS' (Venables & Ripley 2002). An effect was regarded as significant at $P \leq 0.05$.

RESULTS

Due to the poor quality of one IEF-gel, MUP-profile sharing based on Invitrogen IEF gel electrophoresis could only be analyzed for eight of the nine groups. The model was therefore carried out for 120 female dyads originating from eight groups with six females each. In general, protein-bands did not vary greatly and a high number of protein-bands were shared between individuals (see Figure 1). We found no relation between MUP-profile similarity and spatial association in dyads of unfamiliar, unrelated females ($F_{1,111} = 0.055$, $P = 0.8$, see Fig. 2).

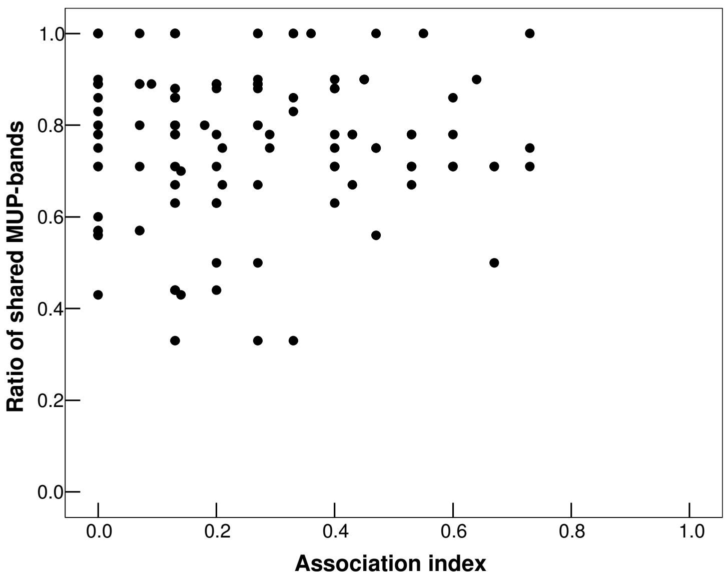


Fig. 2 Relation between MUP-band similarity and spatial association in female dyads.

DISCUSSION

Methodological Problems

In this study we found no indication that MUP profile sharing plays a role in female social partner choice. However, two methodological problems impair the informational value of this experiment.

Gel Electrophoresis

The first and by far biggest problem concerns the method of gel electrophoresis. The main analysis of MUP-band sharing was based on the Invitrogen IEF gels. Even though the

Invitrogen IEF system may be sufficient for certain applications, it should not be the method of choice when an extremely high resolution of protein bands is needed. The problem of insufficient band resolution became obvious when comparing the Invitrogen IEF gel with the Immobiline DryPlate gel (see Fig. 3).

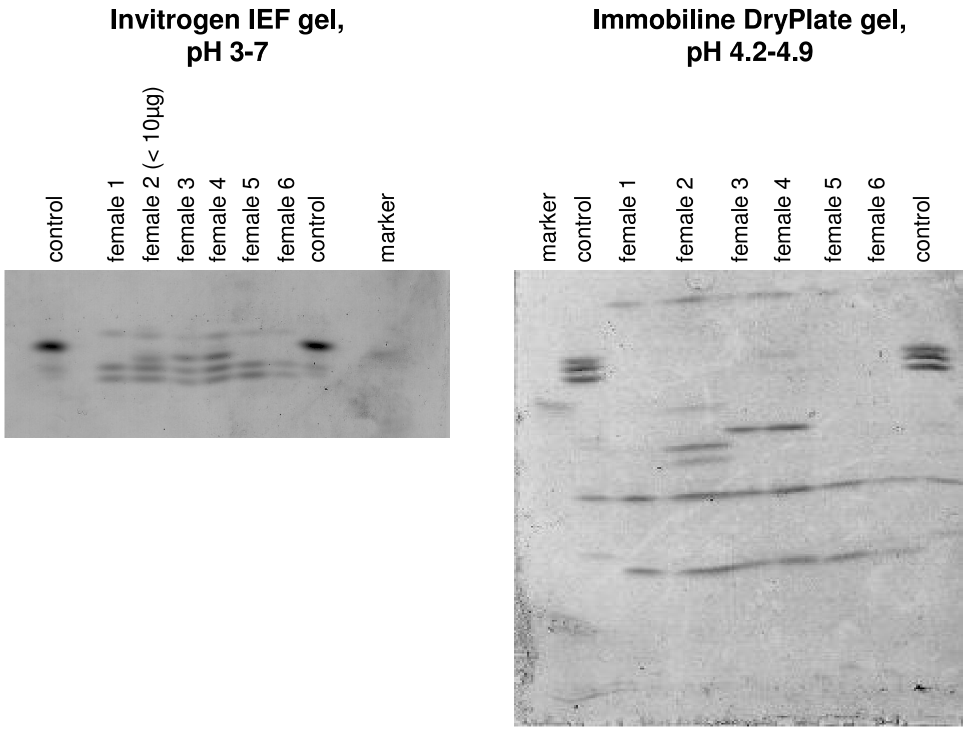


Fig. 3 Comparison of Invitrogen IEF gel (pH 3-7) and Immobiline DryPlate gel (pH 4.2-4.9) for one group of six females. In both systems 10 µg protein was loaded per lane (unless indicated otherwise).

Due to a much smaller pH-range, protein separation and thus MUP-band resolution is significantly better with the Immobiline DryPlate system. In particular for samples with high complexity (e.g. female 2, 3 and 4, see Figure 3), individual differences could only be demonstrated with the Immobiline DryPlate system, but not with the Invitrogen IEF system. According to the Invitrogen IEF System all dyadic comparisons of MUP-band sharing for females 2, 3 and 4 resulted in a ratio of 1.0, which means that their protein bands are assigned as identical. However, using the Immobiline DryPlate system, it became obvious that MUP-bands clearly differed between those individuals (see Figure 3). The low resolution of the Invitrogen IEF system may also explain the seemingly low variance in protein-bands and the generally high proportion of shared MUP-bands between individuals. Unfortunately, most urine samples were used up and the analysis could not be repeated with the Immobiline DryPlate system to verify the results.

The Importance of Male Presence

This experiment was carried out with all-female groups in the absence of males. However, recent data (see chapter 2, this thesis) suggests that male presence significantly alters partner preferences in female house mice. Studies on female social partner choice in the context of reproductive cooperation therefore require the presence of males or male olfactory cues, which was not the case in this experiment.

Overall, the informational value of this analysis should be handled with caution and future experiments on this topic are strongly advised to be carried out in the presence of males, and by using electrophoresis methods with a high resolution of MUP-bands, such as the Immobiline DryPlate system.

MUPs as Potential Cues in Female Social Partner Choice?

In general, females are expected to choose social partners with certain characteristics or strategies. It is suggested, that MUP profiles could be a good candidate for signaling fixed characteristics of an individual, including its propensity to follow a specific behavioural strategy (Rusu et al. in press). For example, in male mice, MUPs are a reliable signal of competitive abilities (Hurst & Beynon 2004; Mucignat-Caretta et al. 2004) and are suggested to have a predictive value for the onset of aggressive behaviour and dispersal tendency (Rusu et al. in press). For female mice, in contrast, we can question weather MUP profiles may signal reproductive abilities or the propensity to communally nurse young. However, no information on this topic is available to date.

In this study, we specifically assumed that similarity in MUP-profiles is used as a cue in social partner choice. This assumption implies that females recognize and choose partners with similar individuality signals, such as for example relatedness, similarity in social status or similar reproductive capacity. Indeed, there are indications that close associations and reproductive cooperation preferentially occur between familiar sisters (König 1997, 2006; Rusu et al. 2004; Rusu & Krackow 2004) and that social partners with low reproductive competition are chosen (Weidt et al. in press), which are presumably similar in social status. However, we do not yet know, if such information is indeed encoded in MUP profiles and if or to which extend female mice perceive and use this information when choosing social partners. Females may rely on other cues, such as MHC (e.g. Manning et al. 1992), behaviour and physiological or hormonal factors, to assess potential social partners.

Clearly, further studies are needed to shed more light on the functional significance of MUPs in the context of female social partner choice.

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correcting literature references and formatting this thesis,
finally finishing what I started some years ago.

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CURRICULUM VITAE

CURRICULUM VITAE

PERSONAL DATA

Surname	Weidt
First name	Andrea
Date of birth	27.09.1974
Nationality	German

EDUCATION

2002-2007	*PhD thesis* PhD student at the Institute of Zoology, Animal Behaviour, University of Zürich, Switzerland. Title of PhD thesis: 'With Whom To Nurse? Conditions and Consequences of Female Social Partner Choice in Wild House Mice', supervised by Prof. Dr. Barbara König.
2000-2001	*Diploma thesis* Diploma student at the Institute of Zoology and Anthropology, University of Göttingen, Germany. Title of Diploma thesis: 'Ecology and Social Behaviour of female Golden Brown Mouse Lemurs (*Microcebus ravelobensis*) in Northwestern Madagascar', supervised by Prof. Dr. Hartmut Rothe and Prof. Dr. Elke Zimmermann (Institute of Zoology, School of Veterinary Medicine, Hannover, Germany).
	Studies in Biology
1996-1998 & 1999-2001	University of Göttingen, Germany
1998-1999	University of Utrecht, The Netherlands.
1994-1996	University of Erlangen, Germany.
1994	*Graduation from High School* Ehrenbürg Gymnasium, Forchheim, Germany.

PUBLICATIONS

Weidt, A.; Hofmann, S.E.; König, B.; 2007: Not only mate choice matters: Fitness consequences of social partner choice in female house mice. Animal Behaviour, in press

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